06-02-00

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Box-Seq

Anticipated Classification of this Application: Class ____ Subclass ____	Prior Application: Examiner S. McGarry Group Art Unit 1635	Attorney's Docket No. 50995-B/JPW/EMW

June 1, 2000

HONORABLE ASSISTANT COMMISSIONER FOR PATENTS
Washington, D.C. 20231

JC822 U.S. PTO 06/01/00

JC542 U.S. PTO 09/585023 06/01/00

SIR:

This is a request for filing a __X__ CONTINUATION ____ DIVISIONAL ____ CONTINUATION-IN-PART application under __X__ 37 C.F.R. §1.53(b) ____ 37 C.F.R. §1.53(d), of pending prior application Serial No. 08/654,482 filed on May 28, 1996 of

Riccardo Dalla-Favera
Inventor(s)

for

IDENTIFICATION OF GENES ALTERED IN MULTIPLE MYELOMA
Title of Invention

1. __X__ Enclosed is a copy of the prior application, as originally filed and an affidavit or declaration verifying it as a true copy.

2. __X__ A verified statement to establish small entity status under 37 C.F.R. §1.9 and 1.27

    ____ is enclosed.

    __X__ was filed in the prior application and such status is still proper and desired (37 C.F.R. §1.28(a)).

3. __X__ The filing fee is calculated as follows:

CLAIMS AS FILED, LESS ANY CLAIMS CANCELLED BY AMENDMENT

	NUMBER FILED		NUMBER EXTRA*		RATE SMALL ENTITY	RATE OTHER ENTITY		FEE SMALL ENTITY	FEE OTHER ENTITY
Total Claims	7 -20	=	0	X	$ 9.00	$18.00	=	$ 0	$
Independent Claims	2 -3	=	0	X	$39.00	$78.00	=	$ 0	$
Multiple Dependent Claims Presented: ____ Yes __X__ No					$130.00	$260.00	=	$ 0	$
					BASIC FEE			$ 345	$ 690
					TOTAL FEE			$345.00	$

*If the different in Col. 1 is less than zero, enter "0" in Col. 2

4. X The Commissioner is hereby authorized to charge payment of the following fees associated with this application or credit any overpayment to Deposit Account No. 03-3125.

X Any additional filing fees required under 37 C.F.R. §1.16.

X Any patent application processing fees under 37 C.F.R. §1.17.

____ The issue fees set forth in 37 C.F.R. §1.18 at or before mailing of the Notice of Allowance, pursuant to 37 C.F.R. §1.311(b).

5. X Three copies of this sheet are enclosed.

6. X A check in the amount of $ 345.00 is enclosed.

7. ____ Cancel claims ____________.

8. ____ Amend the specification by inserting before the first line the sentence: --This is a ___continuation ___division of application Serial No.__________, filed __________.--

9. X 21 Sheet(s) of ___ informal X formal drawing(s) is/are enclosed.

10. ____ Transfer the drawings from the prior application to this application and abandon said prior application as of the filing date accorded this application. A duplicate copy of this sheet is enclosed for filing in the prior application file.

11. ____ Priority of application No. __________ filed on __________ in __________ (country) is claimed under 37 U.S.C. §119.

____ The certified copy of the priority application has been filed in prior application Serial No. __________, filed __________.

12. X The prior application is assigned of record to The Trustees of Columbia University in the City of New York. (a copy of the Assignment is attached)

13. X A preliminary amendment is enclosed.

14. X The power of attorney in the prior application is to:

John P. White (Reg. No. 28,678); Thomas F. Moran (Reg. No. 16,579); Norman H. Zivin (Reg. No. 25,385); Ivan S. Kavrukov (Reg. No. 25,161); Christopher C. Dunham (Reg. No. 22,031); Thomas G. Carulli (Reg. No. 30,616); Robert D. Katz (Reg. No. 30,141); Peter J. Phillips (Reg. No. 29,691); Richard S. Milner (Reg. No. 33,970); Albert Wai-Kit Chan (Reg. No. 36,479); Kristina L. Konstas (Reg. No. 37,864); Mary Anne P. Tanner (Reg. No. 40,197); Timothy X. Witkowski (Reg. No. 40,232); and Mary Catherine DiNunzio (Reg. No. 37,306)

Applicants: Riccardo Dalla-Favera
U.S. Serial No.: Not Yet Known
Filed: Herewith
Cont/Div.
Page 3

June 1, 2000

(a) X The power appears in the original papers in the prior application. (a copy of the Declaration is attached)

(b) ____ Since the power does not appear in the original papers, a copy of the power in the prior application is enclosed.

(c) X Address all future communications to: (May only be completed by applicant, or attorney or agent of record.)

John P. White
Cooper & Dunham LLP
1185 Avenue of the Americas
New York, New York 10036

15. X Also enclosed Express Mail Certificate of Mailing No. EJ 807 507 669 US, a loose set of loose figures, Statement in Accordance and a computer diskette of Sequence Listing.

16. X I hereby verify that the attached papers are a true copy of prior application Serial No. 08/654,482 as originally filed on May 28, 1996.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statement and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

June 1, 2000
Date

Signature John P. White, Reg. No. 28,678

___ INVENTOR(S)
___ ASSIGNEE OF COMPLETE INTEREST
x ATTORNEY OR AGENT OF RECORD
___ FILED UNDER 37 C.F.R. §1.34(a)

Address of Signator:

Cooper & Dunham LLP
1185 Avenue of the Americas
New York, New York 10036

Dkt. 50995-B/JPW/EMW

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Riccardo Dalla-Favera

Serial No.: Not Yet Known (Continuation of U.S. Serial No. 08/654,482, filed May 28, 1996)

Filed: Herewith

For: IDENTIFICATION OF GENES ALTERED IN MULTIPLE MYELOMA

1185 Avenue of the Americas
New York, New York 10036
June 1, 2000

Assistant Commissioner for Patents
Washington, D.C. 20231
Box: Patent Application

SIR:

PRELIMINARY AMENDMENT

Please amend the above-identified application as follows:

In the specification:

At page 1, after the title and before line 3, please insert:

--This application is a continuation of U.S. Serial No. 08/654,482, filed May 26, 1996, now allowed, the contents of which are hereby incorporated by reference.--

In the claims:

Please cancel claims 1-87, 93-97 and 99-101 without prejudice to applicant's right to pursue the subject matter of these claims in

a future continuation application.

Please amend claims 89-92 and 98 under 37 C.F.R. §1.121(b) by deleting the bracketed materials and inserting the underlined materials as follows:

--89. (Amended) A purified human MUM-1 protein of claim 88[.], wherein the MUM-1 protein has the same amino acid sequence as shown in Figure 5B (SEQ. ID NO:14).--

--90. (Amended) An antibody directed to [a] the purified MUM-1 protein of claim 89.--

--91. (Amended) An antibody capable of specifically recognizing MUM-1 protein, wherein the MUM-1 protein has the same amino acid sequence as shown in Figure 5B (SEQ. ID NO:14).--

--92. (Amended) [An] The antibody of claim 91, wherein the MUM-1 protein is a human MUM-1 protein.--

--98. (Amended) [An] A monoclonal antibody of [any one of claims] claim 90. [, 91 and 92.]--

Please add new claims 102-103 as follows:

--102. (New) A monoclonal antibody of claim 91.--

--103. (New) A monoclonal antibody of claim 92.--

REMARKS

Claims 1-101 were pending in the subject application. Applicant has hereinabove canceled claims 1-87, 93-97 and 99-101 without disclaimer or prejudice to applicant's right to pursue the subject matter of these claims at a later date. Applicant has also amended claims 89-92 and 98 and added new claims 102-103. Support for amended claims 89 and 91 may be found in the specification _inter alia_ on page 21, lines 2-4 and Figure 5B. Support for amended claim 92 may be found in the specification _inter alia_ on page 32, lines 10-12 and page 21, lines 2-4. Support for amended claim 98 may be found in the specification _inter alia_ on page 32, lines lines 22-23. Support for new claims 102-103 may be found in the specification _inter alia_ on page 32, lines 23-27 and in previously pending claim 98. Applicant maintains that the amendments are fully supported by the specification and do not raise any issue of new matter. Accordingly, applicant respectfully requests that the Examiner enter the Amendment. Upon entry of the Amendment, claims 88-92 and 98, as amended, and new claims 102-103 will be pending and under examination.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicant's undersigned attorney invites the Examiner to telephone at the number provided

below.

No fee is deemed necessary in connection with the filing of this Preliminary Amendment. However, if any additional fee is required, authorization is hereby given to charge the amount of such fee to Deposit Account No. 03-3125.

Respectfully submitted

John P. White
Registration No. 28,678
Attorney for the Applicant
Cooper & Dunham LLP
1185 Avenue of the Americas
New York, New York 10036
(212) 278-0400

Application for United States Letters Patent

To all whom it may concern:

Be it known that I, Riccardo Dalla-Favera

have invented certain new and useful improvements in

IDENTIFICATION OF GENES ALTERED IN MULTIPLE MYELOMA

of which the following is a full, clear and exact description.

IDENTIFICATION OF GENES ALTERED IN MULTIPLE MYELOMA

The invention disclosed herein was made with Government support under NIH Grant No. CA 44025. Accordingly, the U.S. Government has certain rights in this invention.

Background of the Invention

Throughout this application, various references are referred to within parentheses. Disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains. Full bibliographic citation for these references may be found at the end of this application, preceding the claims.

Multiple myeloma (MM) is an incurable B cell tumor affecting B cell end-stage differentiation. Clinically, the course of MM is similar to end-stage plasma cell leukemia (PCL), i.e., there is an uncontrollable proliferation of myeloma cells accompanied by numerous complications, including hyperviscosity syndromes, hypercalcemia, infections, multiple bone fractures, and organ failure.

Non-random chromosomal translocation is known to play a crucial role in the tumorigenesis of hematologic malignancies (1). In B-cell lymphomas, many important proto-oncogenes deregulated by juxtaposition to immunoglobulin (Ig) gene locus have been identified. Each proto-oncogene is associated with a specific subtype of lymphoma, such as c-MYC in Burkitt's lymphoma, Cyclin DI IBCLI in mantle cell lymphoma, BCL-2 in follicular lymphoma and BCL-6 in diffuse large cell lymphoma (2-8). In contrast, little is known about molecular alterations of

human MM/PCL, due to the difficulty in cytogenetic analysis. However, previous cytogenetic reports have shown a 14q+ chromosome, suggesting the existence of a chromosomal translocation involving the Ig heavy chain (IgH) locus, is observed in 20 ~ 30 % of the MM/PCL cases and it is the most frequent consistent abnormality (9-12). Even in such cases, most cytogenetic data have failed to identify donor chromosomes other than 11q13, 8q24, and 18q21, where proto-oncogenes Cyclin DIIBCL-IIPRADI, c-MYC and BCL-2 are located, respectively. Among them, the 11q13 locus has been demonstrated to be involved in nearly 5~10% of the cases and also in 62% of the established cell lines (13). The t(11;14)(q13;q32) translocation is also accompanied by a corresponding overexpression of the Cyclin D1 gene, which raises a strong possibility of the involvement of this gene, although the breakpoints at 11q13 do not cluster like those of the lymphoma cases (14-16). Recent advances in fluorescence in situ hybridization (FISH) have made it possible to clarify both the frequency of the 14q+ chromosomes and the partner chromosomes of the IgH loci. One such report revealed an intriguing result, i.e., that numerous chromosomal loci are able to translocate to IgH locus, including 6p21, 1q21, 3p11, 7q11, 11q23 (17). This has prompted a search for the proto-oncogenes deregulated by the regulatory elements of the IgH gene for a further understanding of the molecular mechanisms of MM/PCL. In the present study, one candidate proto-oncogene, MUM1 (multiple myeloma oncogene 1), was found juxtaposed to the IgH gene as a result of t(6;14)(p25; q32) translocation in human myeloma cell line, SKMM-1. Over expression of the MUM1 mRNA was observed in this cell line. A second gene, called MUM-2 was found translocated in proximity to the IgH gene on

chromosome 14q32 in human myeloma cell line, U-266.

The method of analysis of 14q+ chromosomal translocations and identification of the genes altered in multiple myeloma of this invention are useful since 1) no method is currently available to determine the chromosomal sequences involved in 14q+ translocations, the most important cytogenetic lesions associated with MM pathogenesis; 2) no specific gene lesion is currently known for MM; 3) no diagnostic method based on gene/DNA lesion is currently available for MM and 4) there are no therapeutic approaches aimed at counteracting the action of abnormal gene products in MM.

Summary of the Invention

This invention provides a method of determining a chromosomal breakpoint in a subject suffering from multiple myeloma which comprises steps of: (a) obtaining a DNA sample from the subject suffering from multiple myeloma; (b) determining whether there is J and C disjunction in the immunoglobulin heavy chain gene in the obtained DNA sample; (c) obtaining a genomic library having clones which contain genomic DNA fragments from the DNA sample which shows positive J and C disjunction; (d) selecting and isolating clones of the obtained library which show positive hybridization with a probe which is capable of specifically hybridizing with the C but not the J region of the immunoglobulin heavy chain gene; (e) preparing fluorescent probes from the genomic DNA fragments of the isolated clones from step (d); (f) hybridizing said fluorescent probes with metaphase chromosomes; and (g) determining the identity of the chromosomes which are capable of hybridizing to said fluorescent probes, wherein the identification of a chromosome other than chromosome 14 would indicate that the chromosomal breakpoint is between chromosome 14 and the identified chromosome, thereby determining a chromosomal breakpoint in a subject suffering from multiple myeloma.

This invention provides a method to identify a gene other than the immunoglobulin gene which is located in chromosome 14, altered by a chromosomal breakpoint detected in a subject suffering from multiple myeloma which comprises steps of: a) selecting a probe having a sequence of a chromosome other than chromosome 14, identified at the chromosomal breakpoint detected in a subject suffering from multiple myeloma, wherein said probe is capable of hybridizing to the unique sequence of the gene other than

the immunoglobulin gene altered by a chromosomal breakpoint detected in a subject suffering from multiple myeloma; b) contacting said probe with mRNA isolated from a cell under conditions permitting formation of a complex between said probe and the mRNA; c) isolating the complex resulting from step (b); d) determining the sequence of the mRNA in the isolated complex, thereby determining the identity of the gene.

This invention provides a gene designated *MUM-1*. This invention provides a gene designated *MUM-2*. This invention provides an isolated nucleic acid molecule encoding a MUM protein. This invention provides a DNA encoding a MUM protein. This invention provides a cDNA encoding a MUM protein. This invention provides a genomic DNA molecule encoding a MUM protein. This invention provides a RNA molecule encoding a MUM protein. This invention provides an isolated nucleic acid molecule encoding a human MUM-1 protein. This invention provides an isolated nucleic acid molecule encoding a human MUM-2 protein. This invention provides an isolated nucleic acid molecule encoding a MUM protein operatively linked to a promoter of RNA transcription. This invention provides a vector comprising the an isolated cDNA encoding a MUM protein. This invention provides a vector which comprises an isolated cDNA encoding a MUM protein. This invention provides a vector which comprises an isolated cDNA encoding a MUM protein, wherein the vector is a plasmid. This invention provides a host cell for the vector which comprises an isolated cDNA encoding a MUM protein.

This invention provides a nucleic acid probe comprising a

nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a MUM protein. This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding a MUM protein.

This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding a MUM protein which is linked to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

This invention provides a nucleic acid probe comprising a the sequence of a nucleic acid molecule encoding a MUM-1 protein which is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14. This invention provides a nucleic acid probe comprising a the sequence of a nucleic acid molecule encoding a MUM-2 protein which is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14.

This invention provides a method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-1 protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-1 protein. This invention provides a method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-2 protein in a sample from a subject which comprises detecting in a sample from the

subject a rearrangement of nucleic acid encoding MUM-2 protein.

This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule encoding a human MUM-1 protein so as to prevent overexpression of the mRNA molecule. This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule encoding a human MUM-2 protein so as to prevent overexpression of the mRNA molecule.

This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to an isolated cDNA molecule encoding a MUM protein. This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to the isolated genomic DNA molecule encoding a MUM protein. This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to an isolated RNA molecule encoding a MUM protein.

This invention provides a purified MUM protein. This invention provides a purified MUM-1 protein. This invention provides an antibody directed to a purified MUM-1 protein. This invention provides an antibody capable of specifically recognizing MUM-1 protein. This invention provides a purified MUM-2 protein. This invention provides an antibody directed to a purified MUM-2 protein. This invention provides an antibody capable of specifically recognizing a MUM-2 protein.

This invention provides a pharmaceutical composition

comprising an amount of an oligonucleotide effective to prevent overexpression of a human MUM-1 protein and a pharmaceutically acceptable carrier capable of passing through a cell membrane. This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide effective to prevent overexpression of a human MUM-2 protein and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

Brief Description of the Figures

Figure 1. JH-Cμ dissociation in *Bam*HI digested DNA of the 14q+ SK-MM-1 cell line. A 10 μ g of the high molecular weight DNA was completely digested with *Bam*HI, loaded on each lane and blotted. The same filter was sequentially hybridized with JH, Cμ, Cγ2, and 0.7B/H probes. JH probe detects two rearranged bands of 12.0 kb and 9.7kb. The 9.7 kb band is comigrated with that probed with Cγ2 probe, suggesting it to be a physiological rearrangement. On the other hand, one allele of the Cμ locus is deleted and another is rearranged (6.5 kb) without being comigrated with rearranged bands of JH. Therefore, 12.0 kb and 6.5 kb bands detected by JH and Cμ (shown by arrowheads) might represent unknown derivative chromosome and derivative 14 chromosome, respectively. As expected, 0.7B/H probe (Fig. 2A) detected the rearranged band comigrated with 6.5kb band of Cμ. Dashed lines show the comigration. Size markers of λ/*Hin*dIII are shown on the left.

Figures 2A-B. Molecular cloning of the breakpoints of the t(6;14) translocation and germline walking at MUM1 locus. (A) Restriction maps of λSKB-4a and λSKS-3 clones representing derivative 6 and 14 are shown, together with germline maps of IgH locus at 14q32 and MUM1

locus at 6p25. Arrows indicate the chromosomal breakpoints. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII. (B) Comparison of the nucleotide sequences around the breakpoints on derivative 6 and derivative 14 chromosome. Homologous regions are indicated by dashes. The arrow indicates the breakpoint. Nucleotide numbers shown below are the same as in the Sμ sequence reported by Sun, et al. (18).

Figure 3. Mapping of the MUM1 locus to chromosome 6p25. λMUM-3 genomic clone (Figure 2A) was used as a probe for *in situ* hybridization. The white arrow indicates the fluorescence signal on chromosome 6 band p25. Right panel shows the G-banding picture stained with DAPI.

Figures 4A-C. Expression of the MUM1 gene in hematopoietic lineage. A 10 ug aliquot of total RNA was loaded on each lane and Northern blot analysis was performed using the 2.1H probe (Figure 2A). GAPDH or β-actin probes were used to control for amount of RNA loaded. (A) MUM1 RNA expression in various hematopoietic cell lines. MUM1 RNA is detected in B cell and mature T cell lines as a single 6kb transcript. HELA, epithelial lineage; LCL, Epstein-Barr virus-transformed lymphoblastoid cell line; RAMOS and SK-MM-1, B-cell lineage; HUT-78 and MOLT-4, T-cell

lineage; HL-60 and U937, myelomonocytic lineage; K562, erythroid lineage. Dashes indicate 28S and 18S. (B) Expression in B cell lines derived from various stages of B cell differentiation. MUM1 RNA is seen throughout the B cell development except for BJAB cell line. 697, pre-B cell stage; RAMOS and BJA-B, Burkitt cell line representing mature-B cell stage; RPMI-8226 and U-266, plasma cell stage. (C) Comparison of the expression level among myeloma cell lines. MUM1 RNA is overexpressed in SK-MM-1 cell line carrying t(6;14). Overexpression of the MUM1 is also demonstrated in XG-4, XG-7, and XG-10 cell lines. RPMI-8226, U-266, EJM, and SKMM-1 are IL-6 (interleukin-6) independent lines, whereas XG-1, XG-2, XG-4, XG-5, XG-6, XG-7, and XG-10 are IL-6 dependent lines.

Figures 5A-B. Sequence of MUM1 cDNA and structure of its predicted protein product. (A) Restriction map of the MUM1 cDNA and the position of the open reading frame (box). The solid box indicates approximate position of the DNA binding domain. Sc, *Sac*II; A, *Apa*I; P, *Pst*I; H, *Hind*III; S, *Sac*I (B) Nucleotide sequence of the MUM1 cDNA and corresponding amino acid sequence. Putative translation initiation codons and preceding stop codons appearing in frame are underlined. The asterisk indicates the translation stop

codon.

Figures 6A-B. Homology between MUM1 and other IRF family proteins. (A) Similarity at N-terminal DNA binding domain. Black background indicates identical residues found more than four times. Gray indicates conserved residues that appear in at least four sequences at a given position. Conserved tryptophan residues in DNA binding domain among IRF family members are indicated by closed circles. (B) Similarity at C-terminal region between human MUM1, Mouse LSIRF/Pip, Human ICSBP, Human ISGF3γ, and Human IRF-3. Black and gray background are as in (A).

Figure 7. Genomic organization of the MUM1 gene and location of the chromosomal breakpoints in multiple myeloma. Filled boxes indicate the coding regions and empty boxes indicate the noncoding regions. The position, and the size of each exon of the MUM1 gene are approximate and have been determined by the hybridizations. One exon in each restriction fragment may consist of more than two exons. Translation initiation codon (ATG) and stop codon (TGA) are indicated. Genomic probes used for further investigations are shown as solid bars below the map. Arrows indicate the chromosomal breakpoints of SKMM-1 cell line and case 10. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII.

Figure 8. Scheme of the t(6;14)(p25;q32) translocation involving the MUM1 and the immunoglobulin heavy chain (IgH) gene loci. VH-D-J-CH indicates variable-diversity joining-constant region of the IgH gene. Direction of the MUM1 gene on the chromosome 6 is tentatively drawn.

Figure 9A-B. Demonstration of JH-Ca disjunction in U-266 cells and cloning of normal and 14q+ chromosomal breakpoints. (A) The panel shows the results of Southern blot analysis of BamHI digested U-266 and normal control (placenta) genomic DNA using the indicated JH and Cα probes. The arrowheads indicate two DNA fragments containing Cα sequences not linked to JH sequences, suggesting the presence of a chromosomal breakpoint in 14q32. (B) The panel provides a schematic representation of the phage clones isolated from a library constructed from U-266 DNA and screened with a Cα probe. Based on restriction enzyme analysis, the three cloned regions represent a normal Cα region (14q32 germ-line), and two rearranged regions (der.14 and 14q32) containing unknown sequences linked to Cα sequences. The 2.5BE probe used for Northern blot analysis of MUM2 transcripts (Fig. 10) is also shown.

Figure 10. Identification of MUM2 RNA transcripts. The

figure shows the results of a Northern blot analysis of RNA extracted from various MM/PCL cell lines using the 2.5BE probe (see Fig. 9) or GAPDH probe (as a control for RNA loading). A 1.9 Kb RNA transcript is detectable in some cell lines including U-266, indicating that the 2.5BE fragments represents part of a gene, MUM2.

Figure 11A-B. Schematic representation of IgH DNA rearrangements in normal B cells and in tumors carrying chromosomal translocations breaking the S region of the IgH locus. Note that in physiological IgH rearrangements (panel 11A) JH sequences and C sequences (Cμ before and Cγ after switch recombination, respectively) are consistently found within the same BamHI restriction fragment. Conversely, JH and C sequences are not linked, and are present on two different chromosomes [derivative X and derivative 14(14q+)] in cells carrying a chromosomal translocation breaking the switch region (panel 11B)

Figure 12A-B. MUM1 cDNA. cDNA insert is cloned into EcoRI/BamHI site of the pBluescript KS+. Bacteria strain used is DH5α cells. pcMUM1.16a contains full length open reading frame of nt. 217-1572.

Figure 13. Breakpoint Cloning of the U-266 Cell Line.

pMUM2-8 has a 22.0 KB insert in BamHI site of pBluescript KS+.

Detailed Description of the Invention

The following standard abbreviations are used throughout the specification to indicate specific nucleotides:

C=cytosine	A=adenosine
T=thymidine	G=guanosine

This invention provides a method of determining a chromosomal breakpoint in a subject suffering from multiple myeloma which comprises steps of: (a) obtaining a DNA sample from the subject suffering from multiple myeloma; (b) determining whether there is J and C disjunction in the immunoglobulin heavy chain gene in the obtained DNA sample; (c) obtaining a genomic library having clones which contain genomic DNA fragments from the DNA sample which shows positive J and C disjunction; (d) selecting and isolating clones of the obtained library which show positive hybridization with a probe which is capable of specifically hybridizing with the C but not the J region of the immunoglobulin heavy chain gene; (e) preparing fluorescent probes from the genomic DNA fragments of the isolated clones from step (d); (f) hybridizing said fluorescent probes with metaphase chromosomes; and (g) determining the identity of the chromosomes which are capable of hybridizing to said fluorescent probes, wherein the identification of a chromosome other than chromosome 14 would indicate that the chromosomal breakpoint is between chromosome 14 and the identified chromosome, thereby determining a chromosomal breakpoint in a subject suffering from multiple myeloma.

In an embodiment, step (b) of the above described method of this invention is performed by Southern blotting. In another embodiment, step (b) of the above method of this

invention is performed by polymerase chain reaction (PCR) with appropriate probes. Polymerase chain reaction is well known in the art. Since the sequences of both the C and J regions of an immunoglobulin heavy chain gene are known, appropriate probes for PCR may routinely be designed.

In an embodiment, the genomic library is a phage vector library. In another embodiment, the genomic DNA fragments are generated by cleaving genomic DNA from cells of the subject with an appropriate restriction enzyme. In a further embodiment, the restriction enzyme is *Bam*HI. In an embodiment, the restriction enzyme is *Sau*3AI. In another embodiment, the probe of step (d) is a human IgH J region JH probe. In a further embodiment, the probe of step (d) is a human IgH Cμ probe. In an embodiment, the probe of step (d) is a human IgH Cγ2 probe. In another embodiment, the chromosomal breakpoint identified is a t(6;14)(p25;q32) translocation. In an embodiment, the chromosomal breakpoint identified is a t(14;15) translocation.

This invention provides a method to identify a gene other than the immunoglobulin gene which is located in chromosome 14, altered by a chromosomal breakpoint detected in a subject suffering from multiple myeloma which comprises steps of: a) selecting a probe having a sequence of a chromosome other than chromosome 14, identified at the chromosomal breakpoint detected in a subject suffering from multiple myeloma, wherein said probe is capable of hybridizing to the unique sequence of the gene other than the immunoglobulin gene altered by a chromosomal breakpoint detected in a subject suffering from multiple myeloma; b) contacting said probe with mRNA isolated from a cell under conditions permitting formation of a complex between said probe and the mRNA; c) isolating the complex resulting from step (b); and d) determining the sequence of the mRNA in the

isolated complex, thereby determining the identity of the gene.

In an embodiment, step (d) of the method to identify a gene other than the immunoglobulin gene which is located in chromosome 14, altered by a chromosomal breakpoint detected in a subject suffering from multiple myeloma comprises steps of: i) synthesizing complementary DNA to the mRNA; and ii) performing sequence analysis of the complementary DNA to determine the sequence of the mRNA.

This invention provides a gene identified by the method to identify a gene other than the immunoglobulin gene which is located in chromosome 14, altered by a chromosomal breakpoint detected in a subject suffering from multiple myeloma.

As used herein, "MUM" means any gene rearranged in 14q+ chromosomal abnormalities associated with multiple myeloma.

This invention provides a gene identified by the above method designated *MUM-1*. This invention provides a gene identified by the above method designated *MUM-2*.

This invention provides a gene identified by the above method, wherein the gene identified comprises a nucleic acid encoding a MUM protein. In an embodiment, the gene identified by the above method comprises a nucleic acid encoding a MUM-1 protein. In another embodiment, the gene identified by the above method comprises a nucleic acid encoding a MUM-2 protein.

This invention provides an isolated nucleic acid molecule encoding a MUM protein. In an embodiment, the isolated

nucleic acid molecule encoding a MUM protein is a DNA molecule. In another embodiment, the isolated nucleic acid molecule encoding a MUM protein is a cDNA molecule.

In an embodiment, a cDNA nucleic acid molecule encoding a MUM-1 protein is cloned into a pBluescript KS+ and the resulting plasmid is designated as pcMUM1-1.6a (ATCC Accession No.). Plasmid pcMUM1-1.6a was deposited on May 28, 1996 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. Plasmid pcMUM1-1.6a was accorded ATCC Accession Number ________.

In another embodiment, a partial cDNA nucleic acid molecule encoding a MUM-1 protein is cloned into a pBluescript KS+ and the resulting plasmid is designated as pMUM1-2.4B/N (ATCC Accession No.). Plasmid pMUM1-2.4B/N was deposited on May 28, 1996 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. Plasmid pMUM1-2.4B/N was accorded ATCC Accession Number ________.

In another embodiment, a partial cDNA nucleic acid molecule encoding a MUM-1 protein is cloned into a pBluescript KS+ and the resulting plasmid is designated as pMUM1-7.7B (ATCC Accession No.). Plasmid pMUM1-7.7B was deposited on May 28, 1996 with the American Type Culture Collection

(ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. Plasmid pMUM1-7.7B was accorded ATCC Accession Number ________.

In another embodiment, a partial cDNA of the nucleic acid molecule encoding a MUM-2 protein is cloned into a pBluescript KS+ and the resulting plasmid is designated as pMUM2-8 (ATCC Accession No.   ). Plasmid pMUM2-8 was deposited on May 28, 1996 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. Plasmid pMUM2-8 was accorded ATCC Accession Number ________.

In an embodiment, the isolated DNA molecule encoding a MUM protein is a cDNA molecule having the nucleotide sequence shown in Figure 5B (SEQ. ID NO).

In an embodiment, the isolated DNA molecule encoding a MUM protein is genomic DNA molecule. In an embodiment, the isolated nucleic acid molecule encoding a MUM protein is an RNA molecule.

In an embodiment, the isolated nucleic acid encodes a human MUM-1 protein. In another embodiment, the isolated nucleic acid molecule encodes a human MUM-2 protein.

In an embodiment, isolated nucleic molecule encodes the a human MUM-1 protein having substantially the same amino acid

sequence as shown in Figure 5B (SEQ. ID NO). In an embodiment, isolated nucleic molecule encodes a human MUM-1 protein having the same amino acid sequence as shown in Figure 5B (SEQ. ID NO). In an embodiment, the isolated nucleic acid molecule encoding a MUM protein is operatively linked to a promoter of RNA transcription.

This invention provides a vector comprising a cDNA molecule encoding a MUM protein. In an embodiment, a vector comprising cDNA encoding for MUM-1 is designated pcMUM1.6a. In an embodiment, a vector comprising partial cDNA encoding for MUM-1 is designated pMUM1.2.4B/N. In an embodiment, a vector comprising partial cDNA encoding for MUM-1 is designated pMUM1-7.7B. In an embodiment, a vector comprising partial cDNA encoding for MUM-2 is designated pMUM2-8. In an embodiment, a vector comprises genomic DNA encoding for MUM. In an embodiment, the vector is a plasmid. In an embodiment, a host cell comprises the vector comprising cDNA encoding for MUM. In an embodiment, a host cell comprises the vector comprising genomic DNA encoding for MUM. In a further embodiment, the host cell comprising vectors comprising cDNA encoding for MUM or comprising genomic DNA encoding for MUM is selected from a group consisting of a bacterial cell, a plant cell, and insect cell and a mammalian cell.

This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a MUM protein. This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the

isolated nucleic acid molecule encoding a MUM protein.

As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs.

In an embodiment, the nucleic acid probe specifically hybridizes with nucleic acid encoding MUM-1. In an embodiment, the nucleic acid probe is complementary to nucleic acid encoding MUM-1. In an embodiment, the nucleic acid probe specifically hybridizes with nucleic acid encoding MUM-2. In an embodiment, the nucleic acid probe is complementary to nucleic acid encoding MUM-2.

In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-1 is a DNA probe. In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-2 is a DNA probe.

In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-1 is a RNA probe. In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-2 is a RNA probe.

In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-1 is a genomic DNA probe. In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-2 is a genomic DNA probe.

In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-1 is labeled with

a detectable marker. In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-2 is labeled with a detectable marker.

In an embodiment, the detectable marker is selected from the group consisting of a radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-1 is linked to a nucleic acid sequence capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule of human chromosome 14. In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-2 is linked to a nucleic acid sequence capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule of human chromosome 14.

This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding a MUM protein which is linked to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

In an embodiment, the nucleic acid probe comprises a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding a MUM-1 protein which is linked to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

In an embodiment, the nucleic acid probe comprises a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding a MUM-2 protein which is linked to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

In an embodiment, the nucleic acid probe comprises a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding a MUM-1 protein which is linked at a specific break point to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

In an embodiment, the nucleic acid probe comprises a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding a MUM-2 protein which is linked at a specific break point to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

In an embodiment, the specific break point of the nucleic acid probe comprises a portion of the t(6;14)(p25;q32) translocation. In an embodiment, the specific break point of the nucleic acid probe comprises a portion of a t(14;15) translocation. In an embodiment, the nucleic acid probe comprising a portion of the t(6;14)(p25;q32) translocation is labeled with a detectable marker. In an embodiment, the nucleic acid probe comprising a portion of a t(14;15) translocation is labeled with a detectable marker. In an

embodiment, the nucleic acid probe comprising a portion of the t(6;14)(p25;q32) or comprising a portion of a t(14;15) translocation of claim 60, has a detectable marker selected from the group consisting of a radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

This invention provides a method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-1 protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-1 protein.

This invention provides a method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-2 protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-2 protein.

In an embodiment, the rearrangement of nucleic acid encoding MUM-1 protein is detected by contacting the nucleic acid from the sample with a MUM-1 probe under conditions permitting the MUM-1 probe to hybridize with the nucleic acid encoding MUM-1 protein from the sample, thereby detecting the rearrangement of nucleic acid encoding MUM-1 protein in the sample.

In an embodiment, the rearrangement of nucleic acid encoding MUM-2 protein is detected by contacting the nucleic acid from the sample with a MUM-2 probe under conditions permitting the MUM-2 probe to hybridize with the nucleic acid encoding MUM-2 protein from the sample, thereby

detecting the rearrangement of nucleic acid encoding MUM-2 protein in the sample.

In an embodiment, the rearrangement of nucleic acid encoding MUM-1 protein is detected by a MUM-1 probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding MUM-1 protein which is linked to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

In an embodiment, the rearrangement of nucleic acid encoding MUM-2 protein is detected by a the MUM-2 probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding MUM-2 protein which is linked to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 15.

In an embodiment, the MUM-1 probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding MUM-1 protein is linked at a specific break point to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

In an embodiment, the MUM-2 probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding MUM-2 protein is linked at a specific break point to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 15.

In an embodiment, the MUM-1 probe comprises a specific break point comprising a portion of the t(6;14)(p25;q32)

translocation. In an embodiment, the MUM-2 probe comprises a specific break point comprising a portion of a t(14;15) translocation.

In an embodiment, the method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-1 protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-1 protein comprises: a) obtaining DNA from the sample of the subject suffering from multiple myeloma; b) performing a restriction digest of the DNA with a panel of restriction enzymes; c) separating the resulting DNA fragments by size fractionation; d) contacting the resulting DNA fragments with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-1 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-1 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14 and labeled with a detectable marker; e) detecting labeled bands which have hybridized to the nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-1 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-1 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14 to create a unique band pattern specific to the DNA of subjects suffering from multiple myeloma; f) preparing DNA obtained from a sample of a subject for diagnosis by steps (a-e); and g) comparing the detected band pattern specific to the DNA obtained from a sample of subjects suffering from multiple myeloma from step (e) and the DNA obtained from a sample of the subject for diagnosis from step (f) to determine whether the patterns

are the same or different and to diagnose thereby predisposition to multiple myeloma if the patterns are the same.

In an embodiment, the method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-2 protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-2 protein comprises: a) obtaining DNA from the sample of the subject suffering from multiple myeloma; b) performing a restriction digest of the DNA with a panel of restriction enzymes; c) separating the resulting DNA fragments by size fractionation; d) contacting the resulting DNA fragments with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-2 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-2 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14 and labeled with a detectable marker; e) detecting labeled bands which have hybridized to the nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-2 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-2 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14 to create a unique band pattern specific to the DNA of subjects suffering from multiple myeloma; f) preparing DNA obtained from a sample of a subject for diagnosis by steps (a-e); and g) comparing the detected band pattern specific to the DNA obtained from a sample of subjects suffering from multiple myeloma from step (e) and the DNA obtained from a sample of the subject for

diagnosis from step (f) to determine whether the patterns are the same or different and to diagnose thereby predisposition to multiple myeloma if the patterns are the same.

In an embodiment, the size fractionation in step (c) is effected by a polyacrylamide or agarose gel. In an embodiment, the detectable marker is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

In an embodiment, the method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-1 protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-1 protein comprises: a) obtaining RNA from the sample of the subject suffering from multiple myeloma; b) separating the RNA sample by size fractionation; c) contacting the resulting RNA species with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-1 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-1 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14 and labeled with a detectable marker; d) detecting labeled bands which have hybridized to the RNA species to create a unique band pattern specific to the RNA of subjects suffering from multiple myeloma; e) preparing RNA obtained from a sample of a subject for diagnosis by steps (a-d); and f) comparing the detected band pattern specific to the RNA obtained from a sample of subjects suffering from multiple myeloma from step (d) and the RNA obtained from a sample of the subject for diagnosis from

step (f) to determine whether the patterns are the same or different and to diagnose thereby predisposition to multiple myeloma if the patterns are the same.

In an embodiment, the method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-2 protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-2 protein comprises: a) obtaining RNA from the sample of the subject suffering from multiple myeloma; b) separating the RNA sample by size fractionation; c) contacting the resulting RNA species with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-2 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-2 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 15 and labeled with a detectable marker; d) detecting labeled bands which have hybridized to the RNA species to create a unique band pattern specific to the RNA of subjects suffering from multiple myeloma; e) preparing RNA obtained from a sample of a subject for diagnosis by steps (a-d); and f) comparing the detected band pattern specific to the RNA obtained from a sample of subjects suffering from multiple myeloma from step (d) and the RNA obtained from a sample of the subject for diagnosis from step (f) to determine whether the patterns are the same or different and to diagnose thereby predisposition to multiple myeloma if the patterns are the same.

In an embodiment, the size fractionation in step (b) is effected by a polyacrylamide or agarose gel. In an embodiment, the detectable marker is radioactive isotope,

enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

In an embodiment, multiple myeloma associated with the expression of a specific human MUM-1 is diagnosed by the method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-1 protein in a DNA or RNA sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-1 protein.

In an embodiment, multiple myeloma associated with the expression of a specific human MUM-2 is diagnosed by the method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-12 protein in a DNA or RNA sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-2 protein.

This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule encoding a human MUM-1 protein so as to prevent overexpression of the mRNA molecule. This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule encoding a human MUM-2 protein so as to prevent overexpression of the mRNA molecule.

This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to the cDNA molecule encoding a MUM protein. This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to the genomic DNA molecule

encoding a MUM protein. This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to the RNA molecule encoding a MUM protein.

This invention provides a purified MUM protein. This invention provides a purified MUM-1 protein. This invention provides a purified human MUM-1 protein. This invention provides an antibody directed to a purified MUM-1 protein. This invention provides an antibody capable of specifically recognizing MUM-1 protein. In an embodiment, the antibody capable of specifically recognizing MUM-1 protein is a human MUM-1 protein.

This invention provides a purified MUM-2 protein. This invention provides a purified human MUM-2 protein. This invention provides an antibody directed to a purified MUM-2 protein. This invention provides an antibody capable of specifically recognizing MUM-2 protein. In an embodiment, the antibody capable of specifically recognizing MUM-2 protein is a human MUM-2 protein.

In an embodiment, the antibody directed to a purified MUM-1 protein is a monoclonal antibody. In an embodiment, the antibody capable of specifically recognizing MUM-1 protein is a monoclonal antibody. In an embodiment, the antibody capable of specifically recognizing MUM-1 protein is a human MUM-1 protein.

In an embodiment, the antibody directed to a purified MUM-2 protein is a monoclonal antibody. In an embodiment, the antibody capable of specifically recognizing MUM-2 protein is a monoclonal antibody. In an embodiment, the antibody capable of specifically recognizing MUM-2 protein is a human

MUM-2 protein.

This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule encoding a human MUM-1 protein so as to prevent overexpression of the mRNA molecule effective to prevent overexpression of a human MUM-1 protein and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide having a sequence capable of specifically hybridizing to a cDNA molecule encoding a MUM protein effective to prevent overexpression of a human MUM-1 protein and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide having a sequence capable of specifically hybridizing to a genomic DNA molecule effective to prevent overexpression of a human MUM-1 protein and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule encoding a human MUM-2 protein so as to prevent overexpression of the mRNA molecule effective to prevent overexpression of a human MUM-2 protein and a pharmaceutically acceptable carrier capable of passing

through a cell membrane.

This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide having a sequence capable of specifically hybridizing to a cDNA molecule encoding a MUM protein effective to prevent overexpression of a human MUM-2 protein and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide having a sequence capable of specifically hybridizing to a genomic DNA molecule effective to prevent overexpression of a human MUM-2 protein and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

EXPERIMENTAL DETAILS

Materials and Methods

Cell lines. The following myeloma cell lines were used in the present study: SK-MM-1, RPMI-8226, U266, EJM, XG-1, XG-2, XG-4, XG-5, XG-6, XG-7, and XG-10. The RPMI-8226 cell line was obtained through the American Type Culture Collection (ATCC, Rockville, MD). SKMM-1 and U-266 cell lines were gifts from Dr. A. N. Houghton and Dr. K. Nilsson, respectively (18; 12). Characterization of these cell lines were previously reported. Six XG cell lines were gifts from Dr. B. Klein and were cultured in RPMI 1640 containing 10% fetal calf serum (FCS), Sx1O-smol/L 2-ME, and rIL-6(1ng/mL)(13;19). Other myeloma cell lines used were all IL-6 independent. The SK-MM-1 cell line was used to isolate the chromosomal breakpoint carrying the 14q+ chromosome without any information on the donor chromosome. XG-1, XG-2, XG-6, XG-8 cell lines are reported to carry the t(11;14)(q13;q32) translocation. XG-5 cells also share both t(11;14) and t(8;14)(q24;q32).

Southern and Northern blot analyses. Southern blot analysis was performed as previously described (21). Briefly, ten micrograms of high molecular-weight DNA extracted from each cell line was digested to completion with *Bam*HI and *Hin*dIII restriction enzymes, size- fractionated on 0.7% agarose gel, and transferred onto Duralose nitrocellulose membrane (Stratagene) according to the manufacturer's instructions. Blots were hybridized with a random-primed DNA probe and washed at 60°C in 0.2 x SSC and 0.1 % SDS for 5 minutes. Genomic probes used in this study were as follows; human IgH

J region JH probe (6.6kb *Bam*HI-*Hin*dIII fragment) was provided by Dr. J. V. Ravetch, human IgH Cμ probe (1.3 kb *Eco*RI fragment) was provided by Dr. S.J. Korsmeyer. Human IgH region Cγ2 probe was provided by Dr. C. Croce.

Northern blot analysis was performed as described previously (21). Briefly, a 10 μg aliquot of total RNA was loaded on each lane and probed with a 2.1H probe of the MUMI gene (Figure 2A). GAPDH or β-actin probes were used as controls for amount of total RNA.

Genomic library. High molecular-weight DNA of SK-MM-1 cell line was digested completely with *Bam*HI and partially with *Sau*3AI, and size-fractionated by using a low-melting point agarose gel. DNA ranging from 10kb to 23kb were purified and ligated into the *Bam*HI sites of λ-DASH II phage vector (Stratagene, La Jolla, CA). After packaging, 3 x 10^5 and 6 x 10^5 recombinant clones of the *Bam*HI digested library and partially digested library were screened with JH and Cμ probes, respectively. To isolate the germline region of the 6p25 locus, a commercially available human placental library (Stratagene) was screened. Positive clones were mapped with restriction enzymes by partial digestion of the phage DNAs followed by probing with T7 and T3 primers labeled with T4 polynucleokinase and 32p-γATP.

cDNA library. A phage library constructed by oligo-dT and random-priming normal human spleen RNA (Clontech) was screened by 2.1H probe (Figure 2A) to isolate initial MUM1 cDNA clones. After the first round of screening, positive clones were used as probes to walk to the 5' side using the same library. Positive clones were subcloned into

pBluescript and analyzed for mapping and sequencing.

DNA sequencing. DNA sequences were determined by the dideoxy chain termination method and analyzed by an ABI(Applied Biosystems) autosequencer. Deletion mutants for sequencing were prepared using exonuclease III and mung bean nuclease. cDNA sequences were analyzed with the Genetics Computer Group (GCG) programs. Sequence homology searches were carried out through the BLAST E-mail server at the National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD.

Fluorescence in situ hybridization (FISH). Metaphase chromosome from human lymphocytes were prepared. A biotin-labeled probe was prepared by nick-translation using Bio-16-dUTP. Conditions for hybridization and washing were described previously (22).

Experimental Results

IgH gene rearrangement of the SK-MM-I cell line. In *Bam*HI digestion, the JH probe detects two rearranged bands of the size of 12.0 kb and 9.7kb (Fig 1). The 9.7 kb band is comigrated with that probed with Cγ2 probe, suggesting it to be a physiological rearrangement, although this cell line secretes only λ chain. One allele of the Cμ locus is deleted and another is rearranged (6.5 kb) without being comigrated with rearranged bands of JH. Hybridization with a Cα probe showed only the germline band (data not shown). These results suggested the possibility of the chromosomal breakpoint between JH and Cμ locus. Hence, the 12.0 kb and the 6.5 kb bands detected by JH and Cμ were considered to

represent unknown derivative chromosome and derivative 14 chromosome, respectively.

Molecular cloning of the t(6;14)(p25;q32) breakpoint. A genomic library constructed with *Bam*HI complete digestion was screened with a JH probe to isolate the 12.0 kb *Bam*HI band. Another library constructed with *Sau*3AI partial digestion was screened with a Cμ probe to isolate phage clones containing the 6.5 kb *Bam*HI fragment. Two phage clones, λSKB-4a and λ SKS-3, considered to represent the unknown derivative and derivative 14 chromosomes respectively, were obtained (Fig 2A). A 0.7 kb *Bam*HI-*Hin*dIII probe (0.7B/H) of the λSKS-3 was used to confirm the comigration with the rearranged 6.5 kb Cμ band by Southern analysis (Fig 1). The chromosomal origin of the centromeric side of the λSKB-4a and telomeric side of the λSKS-3 were confirmed by hybridization to a somatic cell hybrid DNA panel with a 4.5 kb ApaI fragment(4.5A) and 2.1 kb HindIII(2.1H) probes. Both probes showed positive signals in hybrid cell DNA containing a human chromosome 6 (data not shown). These probes were also used to isolate the germline chromosome 6 region by screening the human placental genomic library. One of the phage clone DNA (λMUM-3) was used as a probe for FISH analysis. It identified the localization of this region to be chromosome 6 short arm p25 (Fig 3). To investigate the precise breakpoint within the IgH gene, a 1.5 kb *Hin*dIII-*Eco*RI fragment of the λSKS-3, containing the breakpoint on derivative 14 chromosome was sequenced. The breakpoint was confirmed to be just 3' to the switch μ(S μ) repetitive sequences (Fig 2B). Nucleotide sequencing of the region around the breakpoints of chromosome 6 and derivative 6

chromosome showed that the chromosomal translocation was reciprocal with minimum deletion of both the IgH and 6p25 sequences.

Transcriptional unit in the vicinity of the 6p25 breakpoint. An attempt to find a functional transcriptional unit in the vicinity of the breakpoints was made. Although a 4.5A probe on derivative 6 chromosome could not detect any transcripts, a 2.1H probe on derivative 14 chromosome detected a single 6 kb transcript in the SK-MM-1 cell line. Accordingly, this gene was designated as *MUM1* (multiple myeloma oncogene 1). The same probe was used to study the expression of the MUM1 gene in various hematopoietic cell lines. The 6 kb message was expressed at high levels in most B cell lines and at low levels in peripheral T cell lines (Fig 4A). Cell lines derived from immature T cells, the myelomonocytic lineage, and erythroid lineage do not seem to express *MUM1*. In B cells, MUM1 appears to be expressed throughout the development from the preB cell stage to the plasma cell stage (Fig 4B). However, some of the Burkitt's lymphoma derived cell lines such as BJA-B did not express this gene (data not shown). The expression level of the MUM1 transcript in myeloma cell lines was also examined (Fig 4C). The SK-MM-1 cell line showed a 7.5-fold overexpression when compared with the other three IL-6 independent cell lines, suggesting a deregulated expression of the translocated allele. It is of interest that the IL-6 dependent XG-4, XG-7, and XG-10 cell lines are also expressing at high levels. Particularly, expression in the XG-7 cell line is 19.9 times the average of the aforementioned control cell lines.

MUMI cDNA cloning, sequencing, and homology search. Human spleen cDNA library was initially screened with a 2.1H probe followed by three times walking to 5' side using cDNA probes. A 5.5kb cDNA, approximately corresponding to the size detected by Northern analysis was isolated. This cDNA contained a 1,353 base pair open reading frame (ORF) and a long 3' untranslated region (Fig 5A). The ORF encodes for a protein of 451 amino acids with a predicted molecular weight of 50 kD (Fig 5B). The putative ATG initiation codon at position 217 has G at the -3 position which corresponds to the Kozak consensus sequence (23). The ORF is preceded by two in-frame stop codons. A database search demonstrated a significant similarity between MUM-1 ORF and the interferon regulatory factor (IRF) family proteins. The NH_2-terminal of the MUM-1 ORF shares a high homology with all of the IRF family proteins which share a characteristic DNA binding motif consisting of the conserved 5 tryptophan residues (Fig 6A). The COOH-terminal also has a high homology with ICSBP (interferon consensus sequence binding protein)(21), ISGF3γ (interferon-stimulated gene factor-3 gamma)(22), and IRF-3 protein (23)(Fig 6B), although it did not have any homologous regions with IRF-1 and IRF-2 protein. The highest similarity (95.1%) and identity (91.8%) were found with a possible mouse homolog, LSIRF (lymphoid specific interferon regulatory factor)/Pip (PU-1 cofactor protein-1)(24,25). A high similarity was found with ICSBP (63.98%), ISGF3γ (55.8%), and IRF3 (50.1%) among the human IRF family protein members. A gene sequence encoding a nearly identical protein was recently deposited in GenBank. This gene, termed ICSAT (interferon consensus sequence binding protein in adult T-cell leukemia cell lines or activated T cells) is likely to be the same gene as MUM1

(26).

Breakpoints at MUM1 locus in multiple myeloma.

In order to analyze the exact location of the SK-MM-1 breakpoint at the 6p25 locus and to explore the frequency of the MUM1 gene involvement in myeloma cases, we walked nearly 55kb in a human placental genomic phage library around the MUM1 gene and determined the rough exon-intron structure as shown in Figure 7 (Fig. 7). The SK-MM-1 breakpoint was located 3' to the last exon, containing **a** poly A additional signal, consistent with an unaltered size of the MUM1 transcript of this cell line in Northern analysis. Seven repeat-free genomic probes shown in Figure 7 have been used to investigate the rearrangement in Southern analyses of the 11 MM cell lines and 18 MM cases. One case (case 10) displayed rearranged bands in *Bam*HI and *Xba*I digests when analyzed using a 0.9A probe located at 3' to the MUM1 gene.

Cloning of the MUM2 locus from the U-266 multiple myeloma cell line.

Using an experimental strategy analogous to the one described for the cloning of the MUM1 gene from the SK-MM-1 cell line, a second genetic locus altered in multiple myeloma (MUM2) was identified by analyzing the U-266 multiple myeloma cell line. Briefly, Southern blot analysis using BamH restiction digestion and various Ig probes showed that U-266 DNA contained two rearranged fragments (shown by arrowheads in Fig. 9) containing Cα sequences and lacking J sequences. These two fragments (der 14 and 14q32 in Fig. 9) were cloned from a genomic library constructed from U-266 DNA along with a normal 14q 32 locus (14q32 germline in Fig. 9). In order to determine whether a gene was located in

proximity to the chromosomal breakpoints in der 14, the 2.5 BE restriction fragment (see Fig. 9), which was at the opposite side of the Ig Ca sequences, was used to probe a Northern blot carrying RNA from various MM cell lines. The results (Fig. 10) showed that a 1.9 kb mRNA was detectable in some of these cell lines including U-266. This result showed that a gene, called MUM2, normally not present within the Ig locus on chromosome 14q32, had been translocated in proximity of the Ig locus in U-266 cells. Since the Ig locus contains strong transcriptional regulatory elements, it is likely that the expression of this gene is deregulated in these cells. The structure of the MUM2 gene and its protein are currently under investigation. The 2.5 BE probe and other probes derived from the der 14 phage can be used to screen MM cases for MUM2 rearrangements as shown for MUM1 (Fig. 7).

Experimental Discussion

Using the experimental strategies used for the identification of the MUM1 and MUM2 genes in the SK-MM-1 and U-266 cell lines, respectively, it is possible to analyze most MM cases and isolate the corresponding genes. The scheme shown in Fig. 11 shows that the physiological IgH gene rearrangements (Fig. 11A) typically maintain linkage of C and J sequences and this linkage becomes detectable by using an appropriate restriction enzyme digestion (BamHI in the example in Fig. 11). Conversely, chromosomal translocations (14q+) affecting the IgH locus on 14q32 lead to breakage of the C-J linkage and the two sets of sequences appear on distinct restriction fragments. (Fig. 11B) Table 1 shows the application of this analysis to a panel of MM

cell lines and biopsies. The results show that at least 65% of cases show breakage of the C-J linkage within Ig J or switch regions. The restiction fragments containing either C or J sequences (R in Table 1) can be cloned as shown for the SK-MM-1 and U-266 cell lines and the genes flanking the chromosomal breakpoints can be used as probes to screen additional MM cases for similar rearrangements, whereas the sequence of the genes can be used to understand the consequences of these genetic lesions in multiple myeloma. Cloning of the chromosomal breakpoints and corresponding genes is currently ongoing for all of the MM cases shown in Table 1.

The method of analysis of 14q+ chromosomal translocations and identification of the genes altered in multiple myeloma of this invention will allow 1) the determination of chromosomal sequences involved in 14q+ translocations, the most important cytogenetic lesion associated with MM pathogenesis elucidation; 2) elucidation of specific gene lesions for MM; 3) a diagnostic method based on gene/DNA lesion and 4) a therapeutic approach aimed at counteracting the action of abnormal gene products.

Table 1. Summary of JH-C breakage analysis in MM cell lines and biopsies (cases). Rearrangement (R) involving physiologic Ig recombinations, i.e. retaining JH-C linkage are marked as R*; rearrangements lacking JH-C linkage, and therefore suggesting a 14q+ chromosomal breakpoint, are marked as **R**. The latter represents candidates for cloning an further analysis.

Cell Line/Case	sIg	JH	Cμ	Cα	Sγ3'	possible breakpoint locus
RPMI-8226	λ	D/D	D/D	G	**R**/G	Sγ
U-266	Eλ	R/D	D/D	**R**/**R**/G	G	Sα
EJM	Gλ	**R**/R*	D/D	G	R*/G	JH~Sμ
XG-1	Aκ	R*/D	D/D	R*	G	ND
XG-2	Gλ	R*/D	D/D	G	R*/G	ND
XG-4	Gκ	R*/**R**	D/D	G	R*/G	JH~Sμ
XG-5	λ	**R**/D	D/D	G	G	JH~Sμ
XG-6	Gλ	R*/**R**	D/D	G	R*/G	JH~Sμ
XG-7	Aκ	R/D	D/D	R/G	**R**/D	Sγ
XG-10	G	R*/**R**	D/D	G	R*/G	JH~Sμ
SK-MM-1	κ	R*/**R**	**R**/D	G	R*/G	JH~Sμ
CASE125		R*	G	G	R*	ND
CASE33		**R**/R*	G	G	**R**/R*	Sγ
CASE34		R*	G	R*	G	ND
CASE93		R*	G	R*	G	ND
CASE91		R*	R*	**R**	G	Sα
CASE128		R*	G	R*	G	ND

R*, comigrated bands with JH; **R**, target bands to isolate; ND, not determined

Possible breakage in switch regions:

Cell Lines	4/11 (**36%**)		
Cases	2/6 (**33%**)	Total 6/17 (**35%**)	

Possible breakage in JH ~ switch regions:

Cell Lines	9/11 (**82%**)		
Cases	2/6 (**33%**)	Total 11/17 (**65%**)	

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: IDENTIFICATION OF GENES ALTERED IN MULTIPLE MYELOMA

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(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Lys Leu Arg Gln Trp Leu Ile Asp Gln Ile Asp Ser Gly Lys Tyr Pro
1               5                   10                  15

Gly Leu Val Trp Glu Asn Glu Glu Lys Ser Ile Phe Arg Ile Pro Trp
            20                  25                  30

Lys His Ala Gly Lys Gln Asp Tyr Asn Arg Glu Glu Asp Ala Ala Leu
        35                  40                  45

Phe Lys Ala Trp Ala Leu Phe Lys Gly Lys Phe Arg Glu Gly Ile Asp
    50                  55                  60

Lys Pro Asp Pro Pro Thr Trp Lys Thr Arg Leu Arg Cys Ala Leu Asn
65                  70                  75                  80

Lys Ser Asn Asp Phe Glu Glu Leu Val Glu Arg Ser Gln Leu Asp Ile
                85                  90                  95

Ser Asp Pro Tyr Lys Val Tyr Arg Ile Val Pro Glu
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Lys Leu Arg Gln Trp Leu Ile Asp Gln Ile Asp Ser Gly Lys Tyr Pro
1               5                   10                  15

Gly Leu Val Trp Glu Asn Glu Glu Lys Ser Val Phe Arg Ile Pro Trp
            20                  25                  30

Lys His Ala Gly Lys Gln Asp Tyr Asn Arg Glu Glu Asp Ala Ala Leu
        35                  40                  45

Phe Lys Ala Trp Ala Leu Phe Lys Gly Lys Phe Arg Glu Gly Ile Asp
    50                  55                  60

Lys Pro Asp Pro Pro Thr Trp Lys Thr Arg Leu Arg Cys Ala Leu Asn
65                  70                  75                  80

Lys Ser Asn Asp Phe Glu Glu Leu Val Glu Arg Ser Gln Leu Asp Ile
                85                  90                  95

Ser Asp Pro Tyr Lys Val Tyr Arg Ile Val Pro Glu
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Arg Met Arg Pro Trp Leu Glu Met Gln Ile Asn Ser Asn Gln Ile Pro
1   5   10   15

Gly Leu Ile Trp Ile Asn Lys Glu Glu Met Ile Phe Gln Ile Pro Trp
            20   25   30

Lys His Ala Ala Lys His Gly Trp Asp Ile Asn Lys Asp Ala Cys Leu
        35   40   45

Phe Arg Ser Trp Ala Ile His Thr Gly Arg Tyr Lys Ala Gly Glu Lys
    50   55   60

Glu Pro Asp Pro Lys Thr Trp Lys Ala Asn Phe Arg Cys Ala Met Asn
65   70   75   80

Ser Leu Pro Asp Ile Glu Glu Val Lys Asp Gln Lys Arg Asn Lys Gly
                85   90   95

Ser Ser Ala Val Arg Val Tyr Arg Met Leu Pro Pro
            100   105

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Arg Met Arg Pro Trp Leu Glu Glu Gln Ile Asn Ser Asn Thr Ile Pro
1   5   10   15

Gly Leu Lys Trp Leu Asn Lys Glu Lys Lys Ile Phe Gln Ile Pro Trp

20 25 30

Met His Ala Ala Arg His Gly Trp Asp Val Glu Lys Asp Ala Pro Leu
35 40 45

Phe Arg Asn Trp Ala Ile His Thr Gly Lys His Gln Pro Gly Val Asp
50 55 60

Lys Pro Asp Pro Lys Thr Trp Lys Ala Asn Phe Arg Cys Ala Met Asn
65 70 75 80

Ser Leu Pro Asp Ile Glu Glu Val Lys Asp Lys Ser Ile Lys Lys Gly
85 90 95

Asn Asn Ala Phe Arg Val Tyr Arg Met Leu Pro Leu
100 105

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 107 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Arg Leu Arg Gln Trp Leu Ile Glu Gln Ile Asp Ser Ser Met Tyr Pro
1 5 10 15

Gly Leu Ile Trp Glu Asn Glu Glu Lys Ser Met Phe Arg Ile Pro Trp
20 25 30

Lys His Ala Gly Lys Gln Asp Tyr Asn Gln Glu Val Asp Ala Ser Ile
35 40 45

Phe Lys Ala Trp Ala Val Phe Lys Gly Lys Phe Lys Glu Gly Asp Lys

50 55 60

Ala Glu Pro Ala Thr Trp Lys Thr Arg Leu Arg Cys Ala Leu Asn Lys
65 70 75 80

Ser Pro Asp Phe Glu Glu Val Thr Asp Arg Ser Gln Leu Asp Ile Ser
85 90 95

Glu Pro Tyr Lys Val Tyr Arg Ile Val Pro Glu
100 105

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Lys Leu Arg Asn Trp Val Val Glu Gln Val Glu Ser Gly Gln Phe Pro
1 5 10 15

Gly Val Cys Trp Asp Asp Thr Ala Lys Thr Met Phe Arg Ile Pro Trp
20 25 30

Lys His Ala Gly Lys Gln Asp Phe Arg Glu Asp Gln Asp Ala Ala Phe
35 40 45

Phe Lys Ala Trp Ala Ile Phe Lys Gly Lys Tyr Lys Glu Gly Asp Thr
50 55 60

Gly Gly Pro Ala Val Trp Lys Thr Arg Leu Arg Cys Ala Leu Asn Lys
65 70 75 80

Ser Ser Glu Phe Lys Glu Val Pro Glu Arg Gly Arg Met Asp Val Ala

85 90 95

Glu Pro Tyr Lys Val Tyr Gln Leu Leu Pro Pro
100 105

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Arg Ile Leu Pro Trp Leu Val Ser Gln Leu Asp Leu Gly Gln Leu Glu
1 5 10 15

Gly Val Ala Trp Val Asn Lys Ser Arg Thr Arg Phe Arg Ile Pro Trp
20 25 30

Lys His Gly Leu Arg Gln Asp Ala Gln Gln Glu Asp Phe Gly Ile Phe
35 40 45

Gln Ala Trp Ala Glu Ala Thr Gly Ala Tyr Val Pro Gly Arg Asp Lys
50 55 60

Pro Asp Leu Pro Thr Trp Lys Arg Asn Phe Arg Ser Ser Ala Leu Asn
65 70 75 80

Arg Lys Glu Gly Leu Arg Leu Ala Glu Asp Arg Ser Lys Asp Pro His
85 90 95

Asp Pro His Lys Ile Tyr Glu Phe Val Asn Ser
100 105

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 95 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Lys Arg Leu Cys Gln Ser Thr Ile Tyr Trp Asp Gly Pro Leu Ala Leu
1               5                   10                  15

Cys Asn Asp Arg Pro Asn Lys Leu Glu Arg Asp Gln Thr Cys Lys Leu
            20                  25                  30

Phe Asp Thr Gln Gln Phe Leu Ser Glu Leu Gln Ala Phe Ala His His
        35                  40                  45

Gly Arg Ser Leu Pro Arg Phe Gln Val Thr Leu Cys Phe Gly Glu Glu
    50                  55                  60

Phe Pro Asp Pro Gln Arg Gln Arg Lys Leu Ile Thr Ala His Val Glu
65                  70                  75                  80

Pro Leu Leu Ala Arg Gln Leu Tyr Tyr Phe Ala Gln Gln Asn Ser
                85                  90                  95
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 95 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Lys Arg Leu Cys Gln Ser Arg Ile Tyr Trp Asp Gly Pro Leu Ala Leu
1 5 10 15

Cys Ser Asp Arg Pro Asn Lys Leu Glu Arg Asp Gln Thr Cys Lys Leu
20 25 30

Phe Asp Thr Gln Gln Phe Leu Ser Glu Leu Gln Val Phe Ala His His
35 40 45

Gly Arg Pro Ala Pro Arg Phe Gln Val Thr Leu Cys Phe Gly Glu Glu
50 55 60

Phe Pro Asp Pro Gln Arg Gln Arg Lys Leu Ile Thr Ala His Val Glu
65 70 75 80

Pro Leu Leu Ala Arg Gln Leu Tyr Tyr Phe Ala Gln Gln Asn Thr
85 90 95

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 96 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Lys Arg Leu Cys Gln Gly Arg Val Phe Cys Ser Gly Asn Ala Val Val
1 5 10 15

Cys Lys Gly Arg Pro Asn Lys Leu Glu Arg Asp Glu Val Val Gln Val
20 25 30

Phe Asp Thr Ser Gln Phe Phe Arg Glu Leu Gln Gln Phe Tyr Asn Ser
35 40 45

Gln Gly Arg Leu Pro Asp Gly Arg Val Val Leu Cys Phe Gly Glu Glu
50 55 60

Phe Pro Asp Met Ala Pro Leu Arg Ser Lys Leu Ile Leu Val Gln Ile
65 70 75 80

Glu Gln Leu Tyr Val Arg Gln Leu Ala Glu Glu Ala Gly Lys Ser Cys
85 90 95

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 96 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gln Arg Leu Cys Pro Ile Pro Ile Ser Trp Asn Ala Pro Gln Ala Pro
1 5 10 15

Pro Gly Pro Gly Pro His Leu Leu Pro Ser Asn Glu Cys Val Glu Leu
20 25 30

Phe Arg Thr Ala Tyr Phe Cys Arg Asp Leu Val Arg Tyr Phe Gln Gly
35 40 45

Leu Gly Pro Pro Pro Lys Phe Gln Val Thr Leu Asn Phe Trp Glu Glu
50 55 60

Ser His Gly Ser Ser His Thr Pro Gln Asn Leu Ile Thr Val Lys Met
65 70 75 80

Glu Gln Ala Phe Ala Arg Tyr Leu Leu Glu Gln Thr Pro Glu Gln Gln
85 90 95

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 100 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Gln Arg Leu Gly His Cys His Thr Tyr Trp Ala Val Ser Glu Glu Leu
1 5 10 15

Leu Pro Asn Ser Gly His Gly Pro Asp Gly Glu Val Pro Lys Asp Lys
20 25 30

Glu Gly Gly Val Phe Asp Leu Gly Pro Phe Ile Val Asp Leu Ile Thr
35 40 45

Phe Thr Glu Gly Ser Gly Arg Ser Pro Arg Tyr Ala Trp Leu Phe Cys
50 55 60

Val Gly Glu Ser Trp Pro Gln Asp Gln Pro Trp Thr Lys Arg Leu Val
65 70 75 80

Met Val Lys Val Val Pro Thr Cys Leu Arg Ala Leu Val Glu Met Ala
85 90 95

Arg Val Gly Gly
100

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5176 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 217..1569

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GCCTGACCAA CATGGTAAAA CCCCATCTCT GCTAAAACTA CAAAAAATTA GCTGGATGTG
60

GTGGCAGGGA ACCTGTCATC CCAGCTAGTT GGGAGACTGA GGCAGGAGAA TCGCTCGATC
120

TTGGGACCCA CCGCTGCCCT CAGCTCCGAG TCCAGGGCGA GTGCAGAGCA CAGCGGGCGG
180

AGGACCCCGG GCGCGGGCGC GGACGGCACG CGGGGC ATG AAC CTG GAG GGC GGC
234
Met Asn Leu Glu Gly Gly
1 5

GGC CGA GGC GGA GAG TTC GGC ATG AGC GCG GTG AGC TGC GGC AAC GGG
282
Gly Arg Gly Gly Glu Phe Gly Met Ser Ala Val Ser Cys Gly Asn Gly
10 15 20

AAG CTC CGC CAG TGG CTG ATC GAC CAG ATC GAC AGC GGC AAG TAC CCC
330
Lys Leu Arg Gln Trp Leu Ile Asp Gln Ile Asp Ser Gly Lys Tyr Pro
25 30 35

GGG CTG GTG TGG GAG AAC GAG GAG AAG AGC ATC TTC CGC ATC CCC TGG
378

Gly Leu Val Trp Glu Asn Glu Glu Lys Ser Ile Phe Arg Ile Pro Trp
40 45 50

AAG CAC GCG GGC AAG CAG GAC TAC AAC CGC GAG GAG GAC GCC GCG CTC
426
Lys His Ala Gly Lys Gln Asp Tyr Asn Arg Glu Glu Asp Ala Ala Leu
55 60 65 70

TTC AAG GCT TGG GCA CTG TTT AAA GGA AAG TTC CGA GAA GGC ATC GAC
474
Phe Lys Ala Trp Ala Leu Phe Lys Gly Lys Phe Arg Glu Gly Ile Asp
75 80 85

AAG CCG GAC CCT CCC ACC TGG AAG ACG CGC CTG CGG TGC GCT TTG AAC
522
Lys Pro Asp Pro Pro Thr Trp Lys Thr Arg Leu Arg Cys Ala Leu Asn
90 95 100

AAG AGC AAT GAC TTT GAG GAA CTG GTT GAG CGG AGC CAG CTG GAC ATC
570
Lys Ser Asn Asp Phe Glu Glu Leu Val Glu Arg Ser Gln Leu Asp Ile
105 110 115

TCA GAC CCG TAC AAA GTG TAC AGG ATT GTT CCT GAG GGA GCC AAA AAA
618
Ser Asp Pro Tyr Lys Val Tyr Arg Ile Val Pro Glu Gly Ala Lys Lys
120 125 130

GGA GCC AAG CAG CTC ACC CTG GAG GAC CCG CAG ATG TCC ATG AGC CAC
666
Gly Ala Lys Gln Leu Thr Leu Glu Asp Pro Gln Met Ser Met Ser His
135 140 145 150

CCC TAC ACC ATG ACA ACG CCT TAC CCT TCG CTC CCA GCC CAG CAG GTT
714
Pro Tyr Thr Met Thr Thr Pro Tyr Pro Ser Leu Pro Ala Gln Gln Val
155 160 165

CAC AAC TAC ATG ATG CCA CCC CTC GAC CGA AGC TGG AGG GAC TAC GTC
762
His Asn Tyr Met Met Pro Pro Leu Asp Arg Ser Trp Arg Asp Tyr Val
170 175 180

CCG GAT CAG CCA CAC CCG GAA ATC CCG TAC CAA TGT CCC ATG ACG TTT
810
Pro Asp Gln Pro His Pro Glu Ile Pro Tyr Gln Cys Pro Met Thr Phe
185 190 195

GGA CCC CGC GGC CAC CAC TGG CAA GGC CCA GCT TGT GAA AAT GGT TGC
858
Gly Pro Arg Gly His His Trp Gln Gly Pro Ala Cys Glu Asn Gly Cys
200 205 210

CAG GTG ACA GGA ACC TTT TAT GCT TGT GCC CCA CCT GAG TCC CAG GCT
906
Gln Val Thr Gly Thr Phe Tyr Ala Cys Ala Pro Pro Glu Ser Gln Ala
215 220 225 230

CCC GGA GTC CCC ACA GAG CCA AGC ATA AGG TCT GCC GAA GCC TTG GCG
954
Pro Gly Val Pro Thr Glu Pro Ser Ile Arg Ser Ala Glu Ala Leu Ala
235 240 245

TTC TCA GAC TGC CGG CTG CAC ATC TGC CTG TAC TAC CGG GAA ATC CTC
1002
Phe Ser Asp Cys Arg Leu His Ile Cys Leu Tyr Tyr Arg Glu Ile Leu
250 255 260

GTG AAG GAG CTG ACC ACG TCC AGC CCC GAG GGC TGC CGG ATC TCC CAT
1050
Val Lys Glu Leu Thr Thr Ser Ser Pro Glu Gly Cys Arg Ile Ser His
265 270 275

GGA CAT ACG TAT GAC GCC AGC AAC CTG GAC CAG GTC CTG TTC CCC TAC
1098
Gly His Thr Tyr Asp Ala Ser Asn Leu Asp Gln Val Leu Phe Pro Tyr
280 285 290

CCA GAG GAC AAT GGC CAC AGG AAA AAC ATT GAG AAC CTG CTG AGC CAC
1146
Pro Glu Asp Asn Gly His Arg Lys Asn Ile Glu Asn Leu Leu Ser His
295 300 305 310

CTG GAG AGG GGC GTG GTC CTC TGG ATG GCC CCC GAC GGG CTC TAT GCG
1194

Leu Glu Arg Gly Val Val Leu Trp Met Ala Pro Asp Gly Leu Tyr Ala
315 320 325

AAA AGA CTG TGC CAG AGC ACG ATC TAC TGG GAC GGG CCC CTG GCG CTG
1242
Lys Arg Leu Cys Gln Ser Thr Ile Tyr Trp Asp Gly Pro Leu Ala Leu
330 335 340

TGC AAC GAC CGG CCC AAC AAA CTG GAG AGA GAC CAG ACC TGC AAG CTC
1290
Cys Asn Asp Arg Pro Asn Lys Leu Glu Arg Asp Gln Thr Cys Lys Leu
345 350 355

TTT GAC ACA CAG CAG TTC TTG TCA GAG CTG CAA GCG TTT GCT CAC CAC
1338
Phe Asp Thr Gln Gln Phe Leu Ser Glu Leu Gln Ala Phe Ala His His
360 365 370

GGC CGC TCC CTG CCA AGA TTC CAG GTG ACT CTA TGC TTT GGA GAG GAG
1386
Gly Arg Ser Leu Pro Arg Phe Gln Val Thr Leu Cys Phe Gly Glu Glu
375 380 385 390

TTT CCA GAC CCT CAG AGG CAA AGA AAG CTC ATC ACA GCT CAC GTA GAA
1434
Phe Pro Asp Pro Gln Arg Gln Arg Lys Leu Ile Thr Ala His Val Glu
395 400 405

CCT CTG CTA GCC AGA CAA CTA TAT TAT TTT GCT CAA CAA AAC AGT GGA
1482
Pro Leu Leu Ala Arg Gln Leu Tyr Tyr Phe Ala Gln Gln Asn Ser Gly
410 415 420

CAT TTC CTG AGG GGC TAC GAT TTA CCA GAA CAC ATC AGC AAT CCA GAA
1530
His Phe Leu Arg Gly Tyr Asp Leu Pro Glu His Ile Ser Asn Pro Glu
425 430 435

GAT TAC CAC AGA TCT ATC CGC CAT TCC TCT ATT CAA GAA TGAAAAATGT
1579
Asp Tyr His Arg Ser Ile Arg His Ser Ser Ile Gln Glu
440 445 450

CAAGATGAGT GGTTTTCTTT TTCCTTTTTT TTTTTTTTTT TTTTGATACG GAGATACGGG 1639

GTCTTGCTCT GTCTCCCAGG CTGGAGTGCA GTGACACAAT CTCAGCTCAC TGTGACCTCC 1699

GCCTCCTGGG TTCAAGAGAC TCTCCTGCCT CAGCCTCCCT GGTAGCTGGG ATTACAGGTG 1759

TGAGCCACTG CACCCACCCA AGACAAGTGA TTTTCATTGT AAATATTTGA CTTTAGTGAA 1819

AGCGTCCAAT TGACTGCCCT CTTACTGTTT TGAGGAACTC AGAAGTGGAG ATTTCAGTTC 1879

AGCGGTTGAG GAGAATTGCG GCGAGACAAG CATGGAAAAT CAGTGACATC TGATTGGCAG 1939

ATGAGCTTAT TTCAAAAGGA AGGGTGGCTT TGCATTTTCT TGTGTTCTGT AGACTGCCAT 1999

CATTGATGAT CACTGTGAAA ATTGACCAAG TGATGTGTTT ACATTTACTG AAATGCGCTC 2059

TTTAATTTGT TGTAGATTAG GTCTTGCTGG AAGACAGAGA AAACTTGCCT TTCAGTATTG 2119

ACACTGACTA GAGTGATGAC TGCTTGTAGG TATGTCTGTG CCATTTCTCA GGGAAGTAAG 2179

ATGTAAATTG AAGAAGCCTC ACACGTAAAA GAAATGTATT AATGTATGTA GGAGCTGCAG 2239

TTCTTGTGGA AGACACTTGC TGAGTGAAGG AAATGAATCT TTGACTGAAG CCGTGCCTGT 2299

AGCCTTGGGG AGGCCCATCC CCCACCTGCC AGCGGTTTCC TGGTGTGGGT CCCTCTGCCC 2359

CACCCTCCTT CCCATTGGCT TTCTCTCCTT GGCCTTTCCT GGAAGCCAGT TAGTAAACTT 2419

CCTATTTTCT TGAGTCAAAA AACATGAGCG CTACTCTTGG ATGGGACATT TTTGTCTGTC 2479

CTACAATCTA GTAATGTCTA AGTAATGGTT AAGTTTTCTT GTTTCTGCAT CTTTTTGACC 2539

CTCATTCTTT AGAGATGCTA AAATTCTTCG CATAAAGAAG AAGAAATTAA GGAACATAAA 2599

TCTTAATACT TGAACTGTTG CCCTTCTGTC CAAGTACTTA ACTATCTGTT CCCTTCCTCT 2659

GTGCCACGCT CCTCTGTTTG TTTGGCTGTC CAGCGATCAG CCATGGCGAC ACTAAAGGAG 2719

GAGGAGCCGG GGACTCCCAG GCTGGAGAGC ACTGCCAGGA CCCACCACTG GAAGCAGGAT 2779

GGAGCTGACT ACGGAACTGC ACACTCAGTG GGCTGTTTCT GCTTATTTCA TCTGTTCTAT 2839

GCTTCCTCGT GCCAATTATA GTTTGACAGG GCCTTAAAAT TACTTGGCTT TTTCCAAATG 2899

CTTCTATTTA TAGAAATCCC AAAGACCTCC ACTTGCTTAA GTATACCTAT CACTTACATT 2959

TTTGTGGTTT TGAGAAAGTA CAGCAGTAGA CTGGGGCGTC ACCTCCAGGC CGTTTCTCAT 3019

ACTACAGGAT ATTTACTATT ACTCCCAGGA TTCAGCAGAA GATTGCGTTA GCTCTCAAAT 3079

GTGTGTTCCT GCTTTTCTAA TGGATATTTT AAATTCATTC AACAAGCACC TAGTAAGTGC 3139

CTGCTGTATC CCTACATTAC ACAGTTCAGC CTTTATCAAG CTTAGTGAGC AGTGAGCACT 3199

GAAACATTAT TTTTTAATGT TTAAAAAGTT TCTAATATTA AAGTCAGAAT ATTAATACAA 3259

TTAATATTAA TATTAACTAC AGAAAAGACA AACAGTAGAG AACAGCAAAA AAATAAAAAG
3319

GATCTCCTTT TTTCCCAGCC CAAATTCTCC TCTCTAAAAG TGTCCACAAG AAGGGGTGTT
3379

TATTCTTCCA ACACATTTCA CTTTTCTGTA AATATACATA AACTTAAAAA GAAAACCTCA
3439

TGGAGTCATC TTGCACACAC TTTTCATGCA GTGCTCTTTG TAGCTAAACA GTGAAGATTT
3499

ACCTCGTTCT GCTCAGAGGC CTTGCTGTGG AGCTCCACTG CCATGTACCC AGTAGGGTTT
3559

GACATTTCAT TAGCCATGCA ACATGGATAT GTATTGGGCA GCAGACTGTG TTTCGTGAAC
3619

TGCAGTGATG TATACATCTT ATAGATGCAA AGTATTTTGG GGTATATTAT CCTAAGGGAA
3679

GATAAAGATG ATATTAAGAA CTGCTGTTTC ACGGGGCCCT TACCTGTGAC CCTCTTTGCT
3739

GAAGAATATT TAACCCCACA CAGCACTTCA AAGAAGCTGT CTTGGAAGTC TGTCTCAGGA
3799

GCACCCTGTC TTCTTAATTC TCCAAGCGGA TGCTCCATTT CAATTGCTTT GTGACTTCTT
3859

CTTCTTTGTT TTTTTAAATA TTATGCTGCT TTAACAGTGG AGCTGAATTT TCTGGAAAAT
3919

GCTTCTTGGC TGGGGCCACT ACCTCCTTTC CTATCTTTAC ATCTATGTGT ATGTTGACTT
3979

TTTAAAATTC TGAGTGATCC AGGGTATGAC CTAGGGAATG AACTAGCTAT GGAAATAACT
4039

CAGGGTTAGG AATCCTAGCA CTTGTCTCAG GACTCTGAAA AGGAACGGCT TCCTCATTCC
4099

TTGTCTTGAT AAAGTGGAAT TGGCAAACTA GAATTTAGTT TGTACTCAGT GGACAGTGCT
4159

GTTGAAGATT TGAGGACTTG TTAAAGAGCA CTGGGTCATA TGGAAAAAAT GTATGTGTCT
4219

CCCCAGGTGC ATTTTCTTGG TTTATGTCTT GTTCTTGAGA TTTTGTATAT TTAGGAAAAC
4279

CTCAAGCAGT AATTAATATC TCCTGGAACA CTATAGAGAA CCAAGTGACC GACTCATTTA
4339

CAACTGAAAC CTAGGAAGCC CCTGAGTCCT GAGCGAAAAC AGGAGAGTTA GTCGCCCTAC
4399

AGAAAACCCA GCTAGACTAT TGGGTATGAA CTAAAAAGAG ACTGTGCCAT GGTGAGAAAA
4459

ATGTAAAATC CTACAGTGGA ATGAGCAGCC CTTACAGTGT TGTTACCACC AAGGGCAGGT
4519

AGGTATTAGT GTTTGAAAAA GCTGGTCTTT GAGCGAGGGC ATAAATACAG CTAGCCCCAG
4579

GGGTGGAACA ACTGTGGGAG TCTTGGGTAC TCGCACCTCT TGGCTTTGTT GATGCTCCGC
4639

CAGGAAGGCC ACTTGTGTGT GCGTGTCAGT TACTTTTTTA GTAACAATTC AGATCCAGTG
4699

TAAACTTCCG TTCATTGCTC TCCAGTCACA TGCCCCCACT TCCCCACAGG TGAAAGTTTT
4759

TCTGAAGTGT TGGGATTGGT TAAGGTCTTT ATTTGTATTA CGTATCTCCC CAAGTCCTCT
4819

GTGGCCAGCT GCATCTGTCT GAATGGTGCG TGAAGGCTCT CAGACCTTAC ACACCATTTT
4879

GTAAGTTATG TTTTACATGC CCCGTTTTTG AGACTGATCT CGATGCAGGT GGATCTCCTT
4939

GAGATCCTGA TAGCCTGTTA CAGGAATGAA GTAAAGGTCA GTTTTTTTTG TATTGATTTT 4999

CACAGCTTTG AGGAACATGC ATAAGAAATG TAGCTGAAGT AGAGGGGACG TGAGAGAAGG 5059

GCCAGGCCGG CAGGCCAACC CTCCTCCAAT GGAAATTCCC GTGTTGCTTC AAACTGAGAC 5119

AGATGGGACT TAACAGGCAA TGGGGTCCAC TTCCCCCTCT TCAGCATCCC CCGTACC 5176

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 451 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Asn Leu Glu Gly Gly Gly Arg Gly Gly Glu Phe Gly Met Ser Ala
1 5 10 15

Val Ser Cys Gly Asn Gly Lys Leu Arg Gln Trp Leu Ile Asp Gln Ile
20 25 30

Asp Ser Gly Lys Tyr Pro Gly Leu Val Trp Glu Asn Glu Glu Lys Ser
35 40 45

Ile Phe Arg Ile Pro Trp Lys His Ala Gly Lys Gln Asp Tyr Asn Arg
50 55 60

Glu Glu Asp Ala Ala Leu Phe Lys Ala Trp Ala Leu Phe Lys Gly Lys
65 70 75 80

Phe Arg Glu Gly Ile Asp Lys Pro Asp Pro Pro Thr Trp Lys Thr Arg
85 90 95

Leu Arg Cys Ala Leu Asn Lys Ser Asn Asp Phe Glu Glu Leu Val Glu
100 105 110

Arg Ser Gln Leu Asp Ile Ser Asp Pro Tyr Lys Val Tyr Arg Ile Val
115 120 125

Pro Glu Gly Ala Lys Lys Gly Ala Lys Gln Leu Thr Leu Glu Asp Pro
130 135 140

Gln Met Ser Met Ser His Pro Tyr Thr Met Thr Thr Pro Tyr Pro Ser
145 150 155 160

Leu Pro Ala Gln Gln Val His Asn Tyr Met Met Pro Pro Leu Asp Arg
165 170 175

Ser Trp Arg Asp Tyr Val Pro Asp Gln Pro His Pro Glu Ile Pro Tyr
180 185 190

Gln Cys Pro Met Thr Phe Gly Pro Arg Gly His His Trp Gln Gly Pro
195 200 205

Ala Cys Glu Asn Gly Cys Gln Val Thr Gly Thr Phe Tyr Ala Cys Ala
210 215 220

Pro Pro Glu Ser Gln Ala Pro Gly Val Pro Thr Glu Pro Ser Ile Arg
225 230 235 240

Ser Ala Glu Ala Leu Ala Phe Ser Asp Cys Arg Leu His Ile Cys Leu
245 250 255

Tyr Tyr Arg Glu Ile Leu Val Lys Glu Leu Thr Thr Ser Ser Pro Glu
260 265 270

Gly Cys Arg Ile Ser His Gly His Thr Tyr Asp Ala Ser Asn Leu Asp
275 280 285

Gln Val Leu Phe Pro Tyr Pro Glu Asp Asn Gly His Arg Lys Asn Ile
290 295 300

Glu Asn Leu Leu Ser His Leu Glu Arg Gly Val Val Leu Trp Met Ala
305 310 315 320

Pro Asp Gly Leu Tyr Ala Lys Arg Leu Cys Gln Ser Thr Ile Tyr Trp
325 330 335

Asp Gly Pro Leu Ala Leu Cys Asn Asp Arg Pro Asn Lys Leu Glu Arg
340 345 350

Asp Gln Thr Cys Lys Leu Phe Asp Thr Gln Gln Phe Leu Ser Glu Leu
355 360 365

Gln Ala Phe Ala His His Gly Arg Ser Leu Pro Arg Phe Gln Val Thr
370 375 380

Leu Cys Phe Gly Glu Glu Phe Pro Asp Pro Gln Arg Gln Arg Lys Leu
385 390 395 400

Ile Thr Ala His Val Glu Pro Leu Leu Ala Arg Gln Leu Tyr Tyr Phe
405 410 415

Ala Gln Gln Asn Ser Gly His Phe Leu Arg Gly Tyr Asp Leu Pro Glu
420 425 430

His Ile Ser Asn Pro Glu Asp Tyr His Arg Ser Ile Arg His Ser Ser
435 440 445

Ile Gln Glu
450

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 152 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TTTTCTCTAC AGTCACCTCC CTGTTTACCA AAGATAATCA CAATAAGTCC AGTTTACTTA
60

CAAAACAAGT TTAGTTATTA GAGGAAACTA AAACTTCAGG ATTCAGTCCA GATAATTTTT
120

AAAAACTCTA AAACAATGGA CAGGGCTAGA AT
152

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 152 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TGGGCTCGGC CTGGTGGGGC AGCCACAGCG GGACGCAGTA GTGAAAGTCC AGTTTACTTA
60

CAAAACAAGT TTAGTTATTA GAGGAAACTA AAACTTCAGG ATTCAGCAGG GCATGAGGAG
120

GCAGCTCCTC ACCCTCCCTT TCTCTTTTGT AC
152

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 152 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TGGGCTCGGC CTTGGTGGGG CAGCCACAGC GGGACGCAAG TAGTGAGGGC ACTCAGAACG
60

CCACTCAGCC CCGACAGGGC ACTCAGAACG CCACTCAGCC CCGACAGGCA GGGCACGAGG
120

AGGCAGCTCC TCACCCTCCC TTTCTCTTTT GT
152

What is claimed is:

1. A method of determining a chromosomal breakpoint in a subject suffering from multiple myeloma which comprises steps of:
   (a) obtaining a DNA sample from the subject suffering from multiple myeloma;
   (b) determining whether there is J and C disjunction in the immunoglobulin heavy chain gene in the obtained DNA sample;
   (c) obtaining a genomic library having clones which contain genomic DNA fragments from the DNA sample which shows positive J and C disjunction;
   (d) selecting and isolating clones of the obtained library which show positive hybridization with a probe which is capable of specifically hybridizing with the C but not the J region of the immunoglobulin heavy chain gene;
   (e) preparing fluorescent probes from the genomic DNA fragments of the isolated clones from step (d);
   (f) hybridizing said fluorescent probes with metaphase chromosomes; and
   (g) determining the identity of the chromosomes which are capable of hybridizing to said fluorescent probes, wherein the identification of a chromosome other than chromosome 14 would indicate that the chromosomal breakpoint is between chromosome 14 and the identified chromosome, thereby determining a chromosomal breakpoint in a subject suffering from multiple myeloma.

2. The method of claim 1, wherein step (b) is performed by Southern blotting.

3. The method of claim 1, wherein step (b) is performed by polymerase chain reaction with appropriate probes.

4. The method of claim 1, wherein the genomic library is a phage vector library.

5. The method of claim 4, wherein the genomic DNA fragments are generated by cleaving genomic DNA from cells of the subject with an appropriate restriction enzyme.

6. The method of claim 5, wherein the restriction enzyme is *Bam*HI.

7. The method of claim 5, wherein the restriction enzyme is *Sau*3AI.

8. The method of claim 1, wherein the probe of step (d) is a human IgH J region JH probe.

9. The method of claim 1, wherein the probe of step (d) is a human IgH Cμ probe.

10. The method of claim 1, wherein the probe of step (d) is a human IgH Cγ2 probe.

11. The method of claim 1, wherein the chromosomal breakpoint identified is a t(6;14)(p25;q32) translocation.

12. The method of claim 1, wherein the chromosomal breakpoint identified is a t(14;15) translocation.

13. A method to identify a gene other than the immunoglobulin gene which is located in chromosome 14, altered by a chromosomal breakpoint detected in a subject suffering from multiple myeloma which comprises steps of:

   a) selecting a probe having a sequence of a chromosome other than chromosome 14, identified at the chromosomal breakpoint detected in a subject suffering from multiple myeloma, wherein said probe is capable of hybridizing to the unique sequence of the gene other than the immunoglobulin gene altered by a chromosomal breakpoint detected in a subject suffering from multiple myeloma;
   b) contacting said probe with mRNA isolated from a cell under conditions permitting formation of a complex between said probe and the mRNA;
   c) isolating the complex resulting from step (b);
   d) determining the sequence of the mRNA in the isolated complex, thereby determining the identity of the gene.

14. The method of claim 13, wherein step (d) comprises steps of:

   i) synthesizing complementary DNA to the mRNA; and
   ii) performing sequence analysis of the complementary DNA to determine the sequence of the mRNA.

15. A gene identified by the method of claim 13.

16. The gene of claim 15 designated *MUM-1*.

17. The gene of claim 15 designated *MUM-2*.

18. The method of claim 13, wherein the gene identified comprises a nucleic acid encoding a MUM protein.

19. The method of claim 18, wherein the MUM protein is MUM-1.

20. The method of claim 18, wherein the MUM protein is MUM-2.

21. An isolated nucleic acid molecule encoding a MUM protein.

22. An isolated nucleic acid molecule of claim 21, wherein the nucleic acid molecule is a DNA molecule.

23. The isolated DNA molecule of claim 21, wherein the DNA molecule is a cDNA molecule.

24. The isolated DNA molecule of claim 21, wherein the DNA molecule is a cDNA molecule having the nucleotide sequence shown in Figure 5B (SEQ. ID NO).

25. The isolated DNA molecule of claim 21, wherein the DNA molecule is genomic DNA molecule.

26. The isolated nucleic acid molecule of claim 21, wherein the nucleic acid molecule is an RNA molecule.

27. An isolated nucleic acid molecule of claim 21, wherein the nucleic acid molecule encodes a human MUM-1 protein.

28. An isolated nucleic acid molecule of claim 21, wherein the nucleic acid molecule encodes a human MUM-2 protein.

29. An isolated nucleic molecule of claim 31, wherein the human MUM-1 protein has substantially the same amino acid sequence as shown in Figure 5B (SEQ. ID NO).

30. An isolated nucleic molecule of claim 31, wherein the human MUM-1 protein has the amino acid sequence as shown in Figure 5B (SEQ. ID NO).

31. An isolated nucleic acid molecule of claim 21 operatively linked to a promoter of RNA transcription.

32. A vector comprising the nucleic acid molecule of claim 21.

33. A vector comprising the nucleic acid molecule of any claim 23.

34. A vector comprising the nucleic acid molecule of claim 25.

35. A vector of claim 36, wherein the vector is a plasmid.

36. The plasmid of claim 35, designated pcMUM1-1.6a (ATCC

Accession No.).

37. The plasmid of claim 35, designated pMUM1-2.4B/N (ATCC Accession No.).

38. The plasmid of claim 35, designated pMUM1-7.7B (ATCC Accession No.).

39. The plasmid of claim 35, designated pcMUM2-8 (ATCC Accession No.).

40. A host cell comprising the vector of claims 32.

41. The host cell of claim 40, wherein the cell is selected from a group consisting of a bacterial cell, a plant cell, and insect cell and a mammalian cell.

42. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a MUM protein.

43. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding a MUM protein.

44. The nucleic acid probe of either of claims 42 or 43, wherein the MUM protein is MUM-1.

45. The nucleic acid probe of either of claim 42 or 43, wherein the MUM protein is MUM-2.

46. A DNA probe of claim 44 or 45.

47. A RNA probe of claim 44 or 45.

48. A genomic DNA probe of claim 44 or 45.

49. A nucleic acid probe of claim 44 or 45 labeled with a detectable marker.

50. The nucleic acid probe of claim 49, wherein the detectable marker is selected from the group consisting of a radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

51. A nucleic acid probe of claim 44, wherein the sequence of a nucleic acid molecule encoding a MUM-1 protein is linked to a nucleic acid sequence capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule of human chromosome 14.

52. A nucleic acid probe of claim 45, wherein the sequence of a nucleic acid molecule encoding a MUM-2 protein is linked to a nucleic acid sequence capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule of human chromosome 14.

53. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule of claim 21 which is linked to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

54. The nucleic acid probe of claim 53, wherein the isolated nucleic acid molecule encodes MUM-1.

55. The nucleic acid probe of claim 53, wherein the isolated nucleic acid molecule encodes MUM-2.

56. The nucleic acid probe of claim 54, wherein the sequence of a nucleic acid molecule encoding a MUM-1 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14.

57. The nucleic acid probe of claim 55, wherein the sequence of a nucleic acid molecule encoding a MUM-2 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14.

58. The nucleic acid probe of claim 56, wherein the specific break point comprises a portion of the t(6;14)(p25;q32) translocation.

59. The nucleic acid probe of claim 57, wherein the specific break point comprises a portion of a t(14;15) translocation.

60. The nucleic acid probe of either of claims 58 or 59 labeled with a detectable marker.

61. The nucleic acid probe of claim 60, wherein the detectable marker is selected from the group consisting of a radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

62. A method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-1

protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-1 protein.

63. A method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-2 protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-2 protein.

64. The method of claim 62, wherein the rearrangement of nucleic acid encoding MUM-1 protein is detected by contacting the nucleic acid from the sample with a MUM-1 probe under conditions permitting the MUM-1 probe to hybridize with the nucleic acid encoding MUM-1 protein from the sample, thereby detecting the rearrangement of nucleic acid encoding MUM-1 protein in the sample.

65. The method of claim 63, wherein the rearrangement of nucleic acid encoding MUM-2 protein is detected by contacting the nucleic acid from the sample with a MUM-2 probe under conditions permitting the MUM-2 probe to hybridize with the nucleic acid encoding MUM-2 protein from the sample, thereby detecting the rearrangement of nucleic acid encoding MUM-2 protein in the sample.

66. The method of claim 64, wherein the MUM-1 probe comprises a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding MUM-1 protein which is linked to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

67. The method of claim 65, wherein the MUM-2 probe

comprises a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding MUM-2 protein which is linked to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 15.

68. The method of claim 66, wherein the nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding MUM-1 protein is linked at a specific break point to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

69. The method of claim 67, wherein the nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding MUM-2 protein is linked at a specific break point to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 15.

70. The method of claim 68, wherein the specific break point comprises a portion of the t(6;14)(p25;q32) translocation.

71. The method of claim 69, wherein the specific break point comprises a portion of a t(14;15) translocation.

72. The method of claim 62, which comprises:

a. obtaining DNA from the sample of the subject suffering from multiple myeloma;

b. performing a restriction digest of the DNA with a panel of restriction enzymes;

c. separating the resulting DNA fragments by size fractionation;

d. contacting the resulting DNA fragments with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-1 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-1 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14 and labeled with a detectable marker;

e. detecting labeled bands which have hybridized to the nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-1 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-1 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14 to create a unique band pattern specific to the DNA of subjects suffering from multiple myeloma;

f. preparing DNA obtained from a sample of a subject for diagnosis by steps (a-e); and

g. comparing the detected band pattern specific to the DNA obtained from a sample of

subjects suffering from multiple myeloma from step (e) and the DNA obtained from a sample of the subject for diagnosis from step (f) to determine whether the patterns are the same or different and to diagnose thereby predisposition to multiple myeloma if the patterns are the same.

73. The method of claim 63, which comprises:

a. obtaining DNA from the sample of the subject suffering from multiple myeloma;

b. performing a restriction digest of the DNA with a panel of restriction enzymes;

c. separating the resulting DNA fragments by size fractionation;

d. contacting the resulting DNA fragments with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-2 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-2 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14 and labeled with a detectable marker;

e. detecting labeled bands which have hybridized to the nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-2

protein, wherein the sequence of a nucleic acid molecule encoding a MUM-2 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14 to create a unique band pattern specific to the DNA of subjects suffering from multiple myeloma;

f. preparing DNA obtained from a sample of a subject for diagnosis by steps (a-e); and

g. comparing the detected band pattern specific to the DNA obtained from a sample of subjects suffering from multiple myeloma from step (e) and the DNA obtained from a sample of the subject for diagnosis from step (f) to determine whether the patterns are the same or different and to diagnose thereby predisposition to multiple myeloma if the patterns are the same.

74. The method of claim 72 or 73, wherein the size fractionation in step (c) is effected by a polyacrylamide or agarose gel.

75. The method of claim 72 or 73, wherein the detectable marker is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

76. A method of claim 62 which comprises:

a. obtaining RNA from the sample of the subject suffering from multiple myeloma;

b. separating the RNA sample by size fractionation;

c. contacting the resulting RNA species with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-1 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-1 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14 and labeled with a detectable marker;

d. detecting labeled bands which have hybridized to the RNA species to create a unique band pattern specific to the RNA of subjects suffering from multiple myeloma;

f. preparing RNA obtained from a sample of a subject for diagnosis by steps (a-d); and

g. comparing the detected band pattern specific to the RNA obtained from a sample of subjects suffering from multiple myeloma from step (d) and the RNA obtained from a sample of the subject for diagnosis from step (f) to determine whether the patterns are the same or different and to diagnose thereby predisposition to multiple myeloma if the patterns are the same.

77. A method of claim 63 which comprises:

a. obtaining RNA from the sample of the subject

suffering from multiple myeloma;

b. separating the RNA sample by size fractionation;

c. contacting the resulting RNA species with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-2 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-2 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 15 and labeled with a detectable marker;

d. detecting labeled bands which have hybridized to the RNA species to create a unique band pattern specific to the RNA of subjects suffering from multiple myeloma;

f. preparing RNA obtained from a sample of a subject for diagnosis by steps (a-d); and

g. comparing the detected band pattern specific to the RNA obtained from a sample of subjects suffering from multiple myeloma from step (d) and the RNA obtained from a sample of the subject for diagnosis from step (f) to determine whether the patterns are the same or different and to diagnose thereby predisposition to multiple myeloma if the patterns are the same.

78. The method of claim 76 or 77, wherein the size

fractionation in step (c) is effected by a polyacrylamide or agarose gel.

79. The method of claim 76 or 77, wherein the detectable marker is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

80. The method of either of claim 72 or 76, wherein multiple myeloma associated with the expression of a specific human MUM-1 is diagnosed.

81. The method of either of claim 73 or 77, wherein multiple myeloma associated with the expression of a specific human MUM-2 is diagnosed.

82. An antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule encoding a human MUM-1 protein so as to prevent overexpression of the mRNA molecule.

83. An antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule encoding a human MUM-2 protein so as to prevent overexpression of the mRNA molecule.

84. An antisense oligonucleotide having a sequence capable of specifically hybridizing to the cDNA molecule of claim 23.

85. An antisense oligonucleotide having a sequence capable of specifically hybridizing to the genomic DNA molecule of claim 29.

86. An antisense oligonucleotide having a sequence capable

of specifically hybridizing to the RNA molecule of claim 30.

87. An purified MUM protein.

88. A purified MUM protein, wherein the MUM protein is MUM-1 protein.

89. A purified human MUM-1 protein of claim 88.

90. An antibody directed to a purified MUM-1 protein.

91. An antibody capable of specifically recognizing MUM-1 protein.

92. An antibody of claim 91, wherein the MUM-1 protein is a human MUM-1 protein.

93. A purified MUM protein, wherein the MUM protein is MUM-2 protein.

94. A purified human MUM-2 protein of claim 93.

95. An antibody directed to a purified MUM-2 protein.

96. An antibody capable of specifically recognizing a MUM-2 protein.

97. An antibody of claim 96, wherein the MUM-2 protein is a human MUM-2 protein.

98. An monoclonal antibody of any one of claims 90, 91 and 92.

99. An monoclonal antibody of any one of claims 95, 96, and 97.

100. A pharmaceutical composition comprising an amount of the oligonucleotide of any one of claims 82, 84, 85 and 81 effective to prevent overexpression of a human MUM-1 protein and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

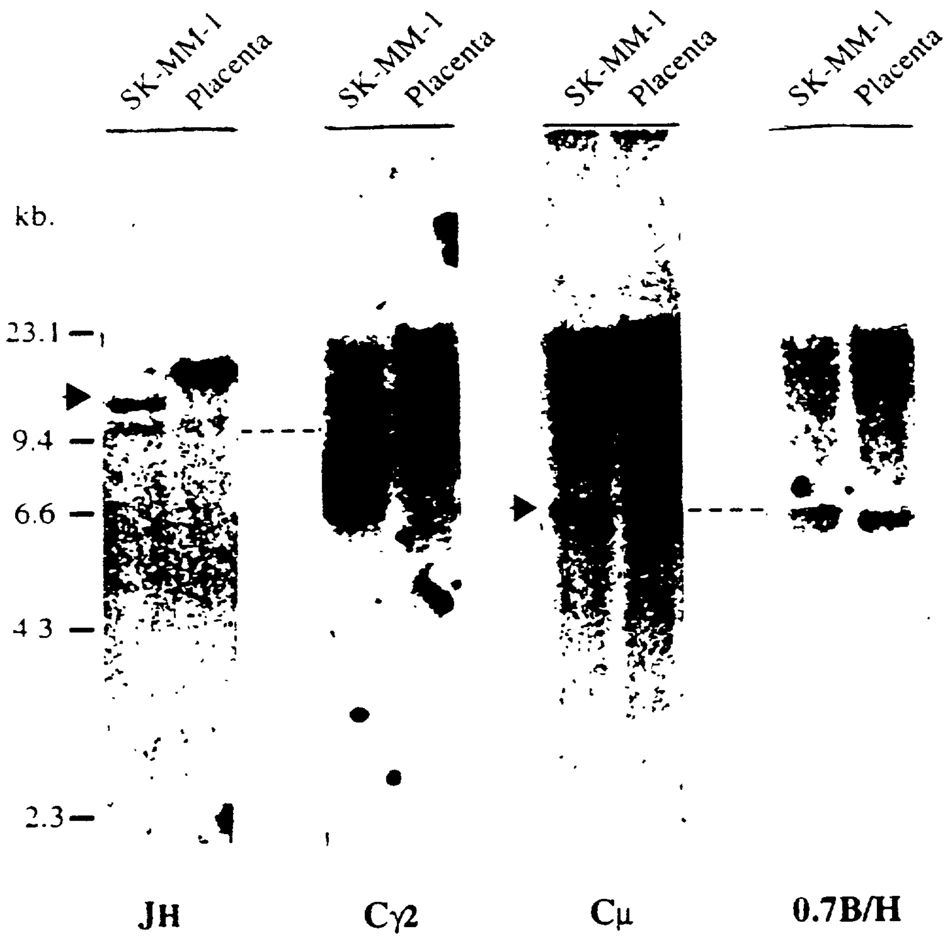
101. A pharmaceutical composition comprising an amount of the oligonucleotide of any one of claims 83, 84, 85 and 81 effective to prevent overexpression of a human MUM-2 protein and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

IDENTIFICATION OF GENES ALTERED IN MULTIPLE MYELOMA

Abstract of the Invention

This invention provides a method of determining a chromosomal breakpoint in a subject suffering from multiple myeloma which comprises steps of: (a) obtaining a DNA sample from the subject suffering from multiple myeloma; (b) determining whether there is J and C disjunction in the immunoglobulin heavy chain gene in the obtained DNA sample; (c) obtaining a genomic library having clones which contain genomic DNA fragments from the DNA sample which shows positive J and C disjunction; (d) selecting and isolating clones of the obtained library which show positive hybridization with a probe which is capable of specifically hybridizing with the C but not the J region of the immunoglobulin heavy chain gene; (e) preparing fluorescent probes from the genomic DNA fragments of the isolated clones from step (d); (f) hybridizing said fluorescent probes with metaphase chromosomes; and (g) determining the identity of the chromosomes which are capable of hybridizing to said fluorescent probes, wherein the identification of a chromosome other than chromosome 14 would indicate that the chromosomal breakpoint is between chromosome 14 and the identified chromosome, thereby determining a chromosomal breakpoint in a subject suffering from multiple myeloma. This invention also provides the identified gene altered by a chromosomal breakpoint and various uses thereof.

FIG. 1


SK-MM-1
Placenta
SK-MM-1
Placenta
SK-MM-1
Placenta
SK-MM-1
Placenta
kb.
23.1
9.4
6.6
4.3
2.3
JH
Cγ2
Cμ
0.7B/H

*Bam*HI

FIG. 2A

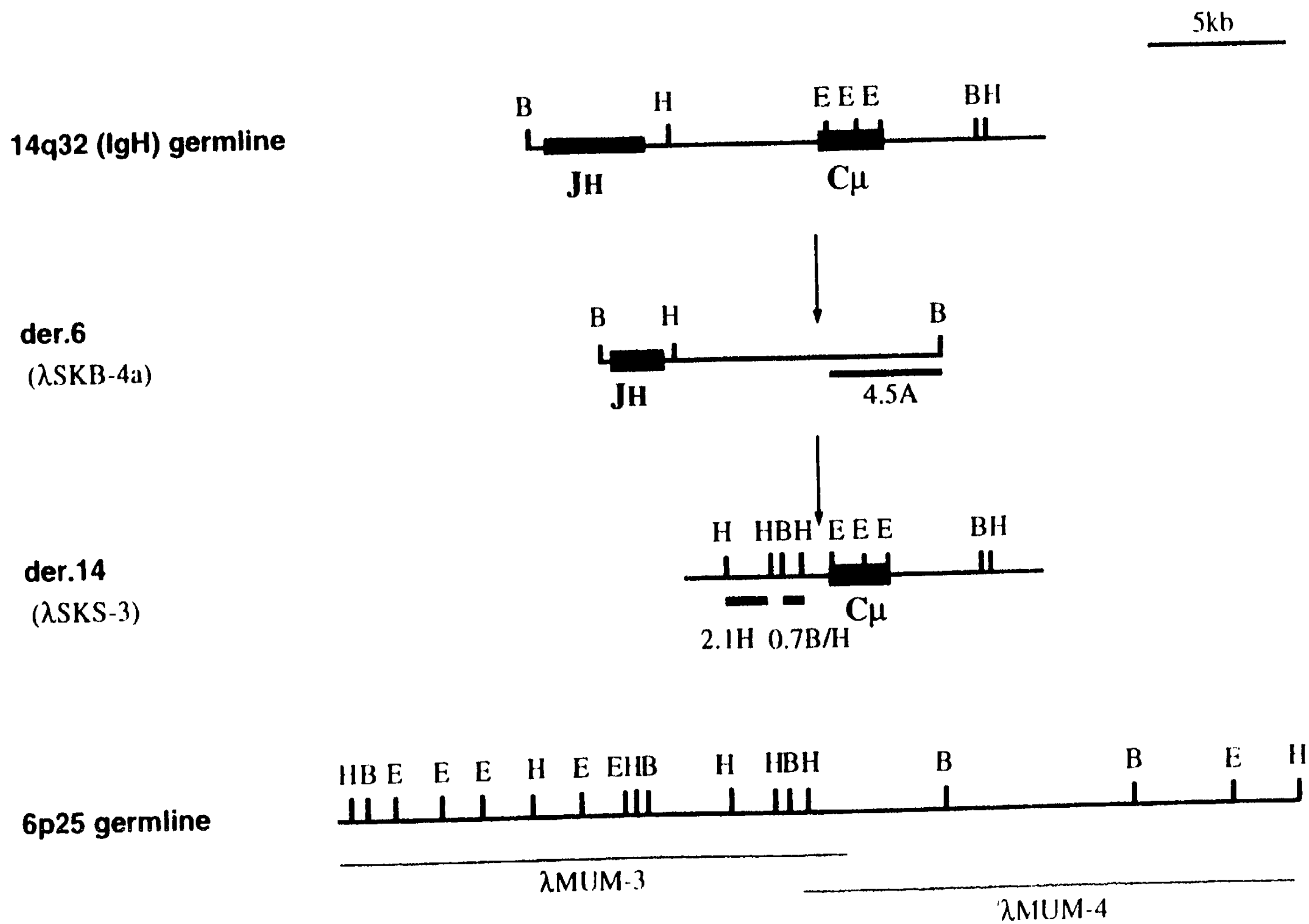

5kb
14q32 (IgH) germline
B
H
E E E
BH
JH
Cμ
der.6
(λSKB-4a)
B
H
B
JH
4.5A
der.14
(λSKS-3)
H
HBH
E E E
BH
Cμ
2.1H 0.7B/H
6p25 germline
HB E
E
E
H
E
EHB
H
HBH
B
B
E
H
λMUM-3
λMUM-4

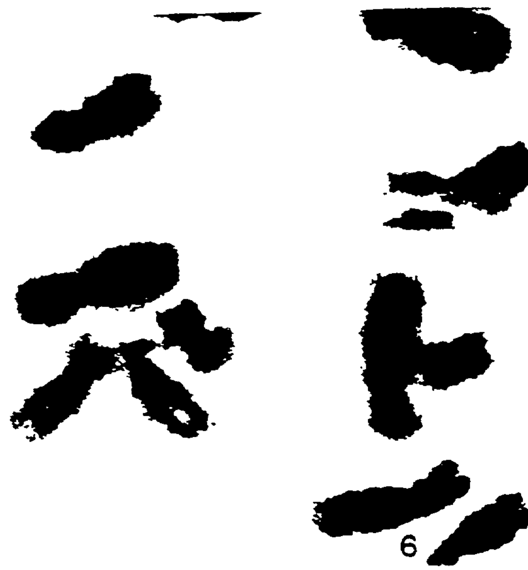
FIG. 2B

```
.6  TTTTCTCTCTACAGTCACCTCCCTGTTTACCAAAGATAATCACAATAAGTCCAGTTTACTTACAAAACAAGTTTAGT
                                               |||||||||||||||||||||||||||||||
.6  TGGGCTCGGCC-TGGTGGGGCAGCCACAGCGGGACGC-AGTAGTGAAAGTCCAGTTTACTTACAAAACAAGTTTAGT
    ||||||||||| ||||||||||||||||||||||||| ||||||||
14  TGGGCTCGGCCTTGGTGGGGCAGCCACAGCGGGACGCAGTAGTGAGGGCACTCAGAACGCCACTCAGCCCCGACAG
      4400      4410      4420      4430      4440      4450      4460      4470

.6  TATTAGAGGAAACTAAAACTTCAGGATTCAGTCCAGATAATTTTTAAAAACTCTAAAACAATGGACAGGGCTAGAAT
    ||||||||||||||||||||||||||||||
.14 TATTAGAGGAAACTAAAACTTCAGGATTCAGCAGGGCATGAGGAGGCAGCTCCTCACCCTCCCTTTCTCTTTTGTAC
                                   |||||||| ||||||||||||||||||||||||||||||||||||
.14 GGCACTCAGAACGCCACTCAGCCCCGACAGGCAGGGCACGAGGAGGCAGCTCCTCACCCTCCCTTTCTCTTTTGT--
        4450      4460      4470      4480      4490      4500      4510
```

FIG. 3A

FIG. 3B



λMUM-3

FIG. 4A

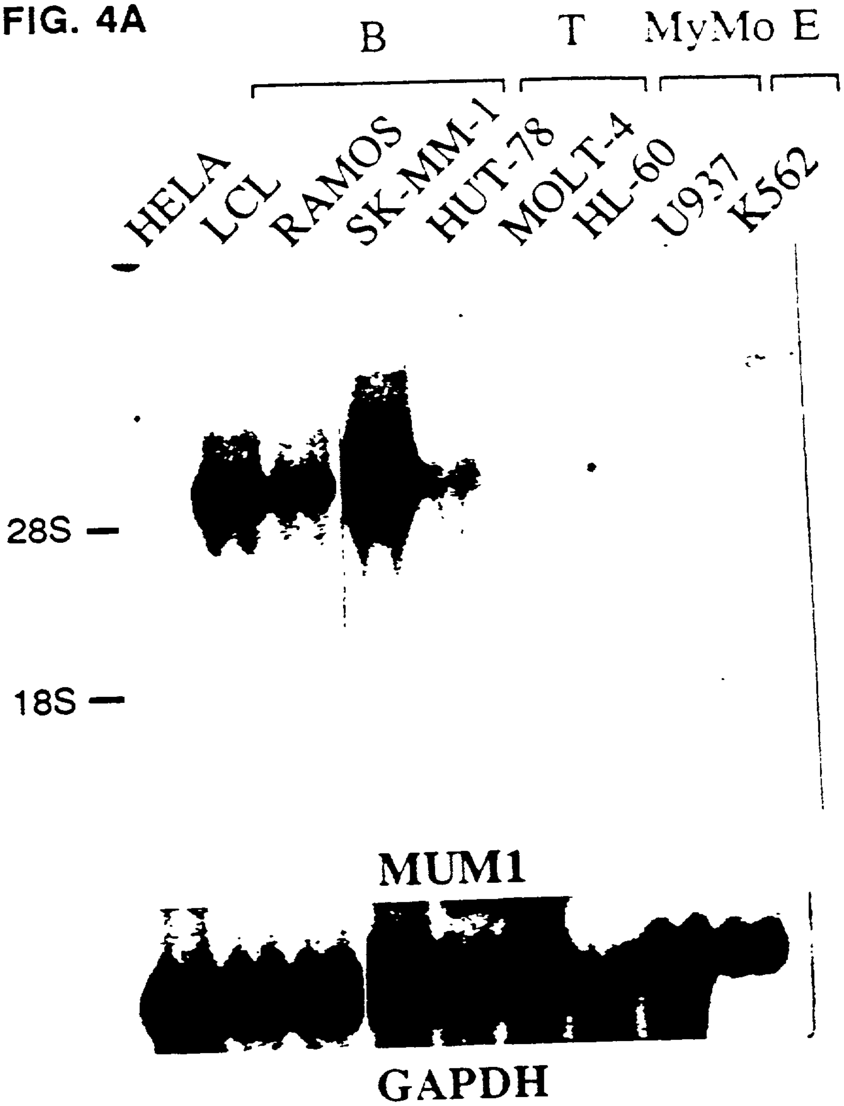

B
T
MyMo
E
HELA
LCL
RAMOS
SK-MM-1
HUT-78
MOLT-4
HL-60
U937
K562
28S
18S
MUM1
GAPDH

FIG. 4B

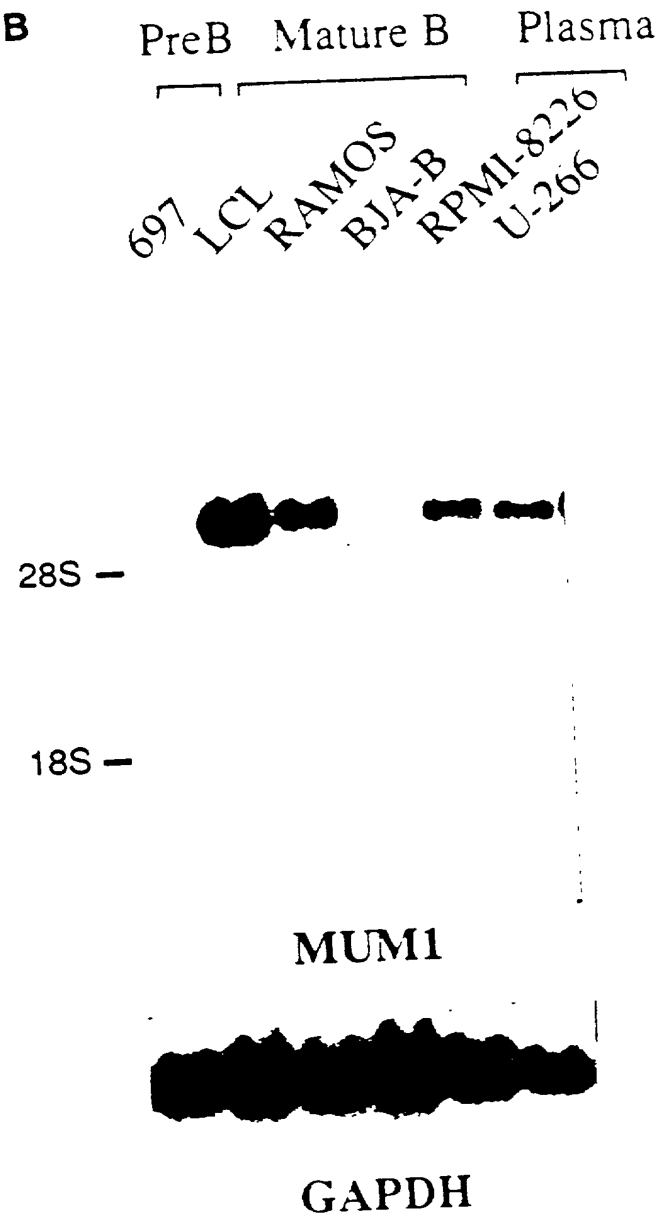

PreB
Mature B
Plasma
697
LCL
RAMOS
BJA-B
RPMI-8226
U-266
28S
18S
MUM1
GAPDH

FIG. 4C

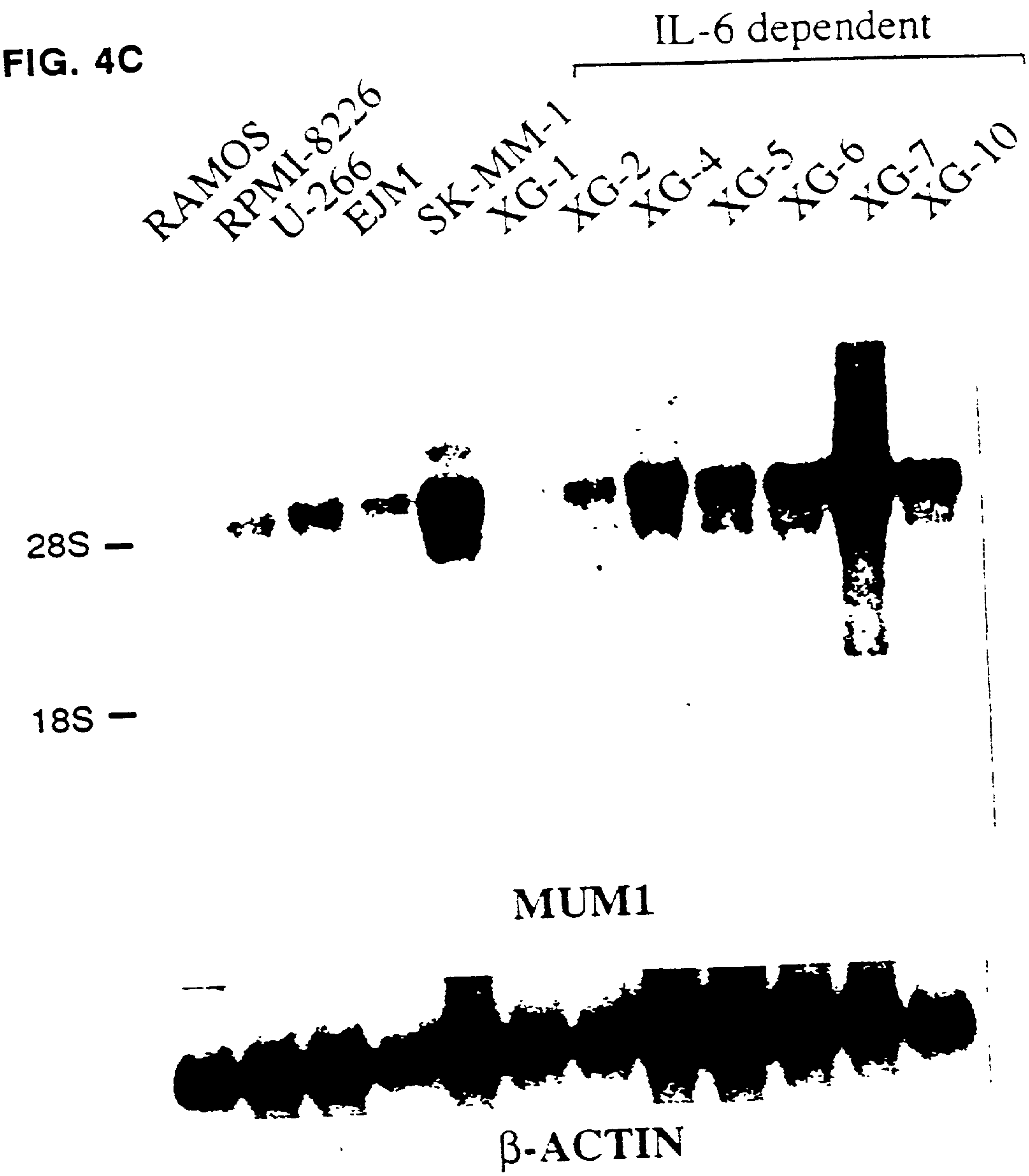

IL-6 dependent
RAMOS
RPMI-8226
U-266
EJM
SK-MM-1
XG-1
XG-2
XG-4
XG-5
XG-6
XG-7
XG-10
28S
18S
MUM1
β-ACTIN

FIG. 5A

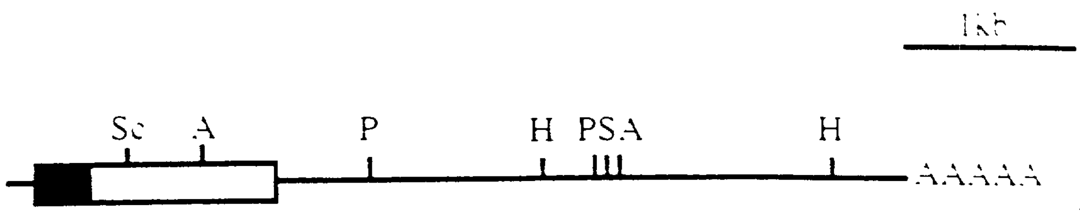


FIG. 5B-1

```
   1  GCCTGACCAA CATGGTAAAA CCCCATCTCT GCTAAAACTA CAAAAAAT
  51  GCTGGATGTG GTGGCAGGGA ACCTGTCATC CCAGCTAGTT GGGAGACT
 101  GGCAGGAGAA TCGCTCGATC TTGGGACCCA CCGCTGCCCT CAGCTCCG
 151  TCCAGGGCGA GTGCAGAGCA CAGCGGGCGG AGGACCCCGG GCGCGGGC
 201  GGACGGCACG CGGGGCATGA ACCTGGAGGG CGGCGGCCGA GGCGGAGA
                        M  N   L  E  G   G  G  R   G  G  E
 251  TCGGCATGAG CGCGGTGAGC TGCGGCAACG GGAAGCTCCG CCAGTGGC
        G  M  S   A  V  S   C  G  N  G   K  L  R   Q  W  L
 301  ATCGACCAGA TCGACAGCGG CAAGTACCCC GGGCTGGTGT GGGAGAAC
       I  D  Q  I   D  S  G   K  Y  P   G  L  V  W   E  N
 351  GGAGAAGAGC ATCTTCCGCA TCCCCTGGAA GCACGCGGGC AAGCAGGA
        E  K  S   I  F  R  I   P  W  K   H  A  G   K  Q  D
 401  ACAACCGCGA GGAGGACGCC GCGCTCTTCA AGGCTTGGGC ACTGTTTA
        N  R  E   E  D  A   A  L  F  K   A  W  A   L  F  K
 451  GGAAAGTTCC GAGAAGGCAT CGACAAGCCG GACCCTCCCA CCTGGAAG
       G  K  F  R   E  G  I   D  K  P   D  P  P  T   W  K
 501  GCGCCTGCGG TGCGCTTTGA ACAAGAGCAA TGACTTTGAG GAACTGGT
        R  L  R   C  A  L  N   K  S  N   D  F  E   E  L  V
 551  AGCGGAGCCA GCTGGACATC TCAGACCCGT ACAAAGTGTA CAGGATTG
        R  S  Q   L  D  I   S  D  P  Y   K  V  Y   R  I  V
 601  CCTGAGGGAG CCAAAAAAGG AGCCAAGCAG CTCACCCTGG AGGACCCG
       P  E  G  A   K  K  G   A  K  Q   L  T  L  E   D  P
 651  GATGTCCATG AGCCACCCCT ACACCATGAC AACGCCTTAC CCTTCGCT
        M  S  M   S  H  P  Y   T  M  T   T  P  Y   P  S  L
 701  CAGCCCAGCA GGTTCACAAC TACATGATGC CACCCCTCGA CCGAAGCT
        A  Q  Q   V  H  N   Y  M  M  P   P  L  D   R  S  W
 751  AGGGACTACG TCCCGGATCA GCCACACCCG GAAATCCCGT ACCAATGT
       R  D  Y  V   P  D  Q   P  H  P   E  I  P  Y   Q  C
 801  CATGACGTTT GGACCCCGCG GCCACCACTG GCAAGGCCCA GCTTGTGA
        M  T  F   G  P  R  G   H  H  W   Q  G  P   A  C  E
 851  ATGGTTGCCA GGTGACAGGA ACCTTTTATG CTTGTGCCCC ACCTGAGT
        G  C  Q   V  T  G   T  F  Y  A   C  A  P   P  E  S
 901  CAGGCTCCCG GAGTCCCCAC AGAGCCAAGC ATAAGGTCTG CCGAAGCC
       Q  A  P  G   V  P  T   E  P  S   I  R  S  A   E  A
 951  GGCGTTCTCA GACTGCCGGC TGCACATCTG CCTGTACTAC CGGGAAAT
        A  F  S   D  C  R  L   H  I  C   L  Y  Y   R  E  I
1001  TCGTGAAGGA GCTGACCACG TCCAGCCCCG AGGGCTGCCG GATCTCCC
        V  K  E   L  T  T   S  S  P  E   G  C  R   I  S  H
1051  GGACATACGT ATGACGCCAG CAACCTGGAC CAGGTCCTGT TCCCCTAC
       G  H  T  Y   D  A  S   N  L  D   Q  V  L  F   P  Y
1101  AGAGGACAAT GGCCACAGGA AAAACATTGA GAACCTGCTG AGCCACCT
        E  D  N   G  H  R  K   N  I  E   N  L  L   S  H  L
```

FIG. 5B-2

```
1151  AGAGGGGCGT GGTCCTCTGG ATGGCCCCG ACGGGCTCTA TGCGAAAA
        R  G  V   V  L  W   M  A  P  D   G  L  Y   A  K  R
1201  CTGTGCCAGA GCACGATCTA CTGGGACGGG CCCCTGGCGC TGTGCAAC
      L  C  Q  S   T  I  Y   W  D  G   P  L  A  L   C  N
1251  CCGGCCCAAC AAACTGGAGA GAGACCAGAC CTGCAAGCTC TTTGACAC
       R  P  N   K  L  E  R   D  Q  T   C  K  L   F  D  T
1301  AGCAGTTCTT GTCAGAGCTG CAAGCGTTTG CTCACCACGG CCGCTCCC
        Q  F  L   S  E  L   Q  A  F  A   H  H  G   R  S  L
1351  CCAAGATTCC AGGTGACTCT ATGCTTTGGA GAGGAGTTTC CAGACCCT
      P  R  F  Q   V  T  L   C  F  G   E  E  F  P   D  P
1401  GAGGCAAAGA AAGCTCATCA CAGCTCACGT AGAACCTCTG CTAGCCAG
       R  Q  R   K  L  I  T   A  H  V   E  P  L   L  A  R
1451  AACTATATTA TTTTGCTCAA CAAAACAGTG GACATTTCCT GAGGGGCT
        L  Y  Y   F  A  Q   Q  N  S  G   H  F  L   R  G  Y
1501  GATTTACCAG AACACATCAG CAATCCAGAA GATTACCACA GATCTATC
      D  L  P  E   H  I  S   N  P  E   D  Y  H  R   S  I
1551  CCATTCCTCT ATTCAAGAAT GAAAAATGTC AAGATGAGTG GTTTTCTT
       H  S  S   I  Q  E  *
1601  TCCTTTTTTT TTTTTTTTTT TTTGATACGG AGATACGGGG TCTTGCTC
1651  TCTCCCAGGC TGGAGTGCAG TGACACAATC TCAGCTCACT GTGACCTC
1701  CCTCCTGGGT TCAAGAGACT CTCCTGCCTC AGCCTCCCTG GTAGCTGG
1751  TTACAGGTGT GAGCCACTGC ACCCACCCAA GACAAGTGAT TTTCATTG
1801  AATATTTGAC TTTAGTGAAA GCGTCCAATT GACTGCCCTC TTACTGTT
1851  GAGGAACTCA GAAGTGGAGA TTTCAGTTCA GCGGTTGAGG AGAATTGC
1901  CGAGACAAGC ATGGAAAATC AGTGACATCT GATTGGCAGA TGAGCTTA
1951  TCAAAAGGAA GGGTGGCTTT GCATTTTCTT GTGTTCTGTA GACTGCCA
2001  ATTGATGATC ACTGTGAAAA TTGACCAAGT GATGTGTTTA CATTTACT
2051  AATGCGCTCT TTAATTTGTT GTAGATTAGG TCTTGCTGGA AGACAGAG
2101  AACTTGCCTT TCAGTATTGA CACTGACTAG AGTGATGACT GCTTGTAG
2151  ATGTCTGTGC CATTTCTCAG GGAAGTAAGA TGTAAATTGA AGAAGCCT
2201  CACGTAAAAG AAATGTATTA ATGTATGTAG GAGCTGCAGT TCTTGTGG
2251  GACACTTGCT GAGTGAAGGA AATGAATCTT TGACTGAAGC CGTGCCTG
2301  GCCTTGGGGA GGCCCATCCC CCACCTGCCA GCGGTTTCCT GGTGTGGG
2351  CCTCTGCCCC ACCCTCCTTC CCATTGGCTT TCTCTCCTTG GCCTTTCC
2401  GAAGCCAGTT AGTAAACTTC CTATTTTCTT GAGTCAAAAA ACATGAGC
2451  TACTCTTGGA TGGGACATTT TTGTCTGTCC TACAATCTAG TAATGTCT
2501  GTAATGGTTA AGTTTTCTTG TTTCTGCATC TTTTTGACCC TCATTCTT
2551  GAGATGCTAA AATTCTTCGC ATAAAGAAGA AGAAATTAAG GAACATAA
2601  CTTAATACTT GAACTGTTGC CCTTCTGTCC AAGTACTTAA CTATCTGT
2651  CCTTCCTCTG TGCCACGCTC CTCTGTTTGT TTGGCTGTCC AGCGATCA
2701  CATGGCGACA CTAAAGGAGG AGGAGCCGGG GACTCCCAGG CTGGAGAG
2751  CTGCCAGGAC CCACCACTGG AAGCAGGATG GAGCTGACTA CGGAACTG
2801  CACTCAGTGG GCTGTTTCTG CTTATTTCAT CTGTTCTATG CTTCCTCG
2851  CCAATTATAG TTTGACAGGG CCTTAAAATT ACTTGGCTTT TTCCAAAT
2901  TTCTATTTAT AGAAATCCCA AAGACCTCCA CTTGCTTAAG TATACCTA
2951  ACTTACATTT TTGTGGTTTT GAGAAAGTAC AGCAGTAGAC TGGGGCGT
```

FIG. 5B-3

```
3051  TCAGCAGAAG ATTGCGTTAG CTCTCAAATG TGTGTTCCTG CTTTTCTA
3101  GGATATTTTA AATTCATTCA ACAAGCACCT AGTAAGTGCC TGCTGTAT
3151  CTACATTACA CAGTTCAGCC TTTATCAAGC TTAGTGAGCA GTGAGCAC
3201  AAACATTATT TTTTAATGTT TAAAAAGTTT CTAATATTAA AGTCAGAA
3251  TTAATACAAT TAATATTAAT ATTAACTACA GAAAAGACAA ACAGTAGA
3301  ACAGCAAAAA AATAAAAAGG ATCTCCTTTT TTCCCAGCCC AAATTCTC
3351  CTCTAAAAGT GTCCACAAGA AGGGGTGTTT ATTCTTCCAA CACATTTC
3401  TTTTCTGTAA ATATACATAA ACTTAAAAAG AAAACCTCAT GGAGTCAT
3451  TGCACACACT TTTCATGCAG TGCTCTTTGT AGCTAAACAG TGAAGATT
3501  CCTCGTTCTG CTCAGAGGCC TTGCTGTGGA GCTCCACTGC CATGTACC
3551  GTAGGGTTTG ACATTTCATT AGCCATGCAA CATGGATATG TATTGGGC
3601  CAGACTGTGT TTCGTGAACT GCAGTGATGT ATACATCTTA TAGATGCA
3651  GTATTTTGGG GTATATTATC CTAAGGGAAG ATAAAGATGA TATTAAGA
3701  TGCTGTTTCA CGGGGCCCTT ACCTGTGACC CTCTTTGCTG AAGAATAT
3751  AACCCCACAC AGCACTTCAA AGAAGCTGTC TTGGAAGTCT GTCTCAGG
3801  CACCCTGTCT TCTTAATTCT CCAAGCGGAT GCTCCATTTC AATTGCTT
3851  TGACTTCTTC TTCTTTGTTT TTTTAAATAT TATGCTGCTT TAACAGTG
3901  GCTGAATTTT CTGGAAAATG CTTCTTGGCT GGGGCCACTA CCTCCTTT
3951  TATCTTTACA TCTATGTGTA TGTTGACTTT TTAAAATTCT GAGTGATC
4001  GGGTATGACC TAGGGAATGA ACTAGCTATG GAAATAACTC AGGGTTAG
4051  ATCCTAGCAC TTGTCTCAGG ACTCTGAAAA GGAACGGCTT CCTCATTC
4101  TGTCTTGATA AAGTGGAATT GGCAAACTAG AATTTAGTTT GTACTCAG
4151  GACAGTGCTG TTGAAGATTT GAGGACTTGT TAAAGAGCAC TGGGTCAT
4201  GGAAAAAATG TATGTGTCTC CCCAGGTGCA TTTTCTTGGT TTATGTCT
4251  TTCTTGAGAT TTTGTATATT TAGGAAAACC TCAAGCAGTA ATTAATAT
4301  CCTGGAACAC TATAGAGAAC CAAGTGACCG ACTCATTTAC AACTGAAA
4351  TAGGAAGCCC CTGAGTCCTG AGCGAAAACA GGAGAGTTAG TCGCCCTA
4401  GAAAACCCAG CTAGACTATT GGGTATGAAC TAAAAAGAGA CTGTGCCA
4451  GTGAGAAAAA TGTAAAATCC TACAGTGGAA TGAGCAGCCC TTACAGTG
4501  GTTACCACCA AGGGCAGGTA GGTATTAGTG TTTGAAAAAG CTGGTCTT
4551  AGCGAGGGCA TAAATACAGC TAGCCCCAGG GGTGGAACAA CTGTGGGA
4601  CTTGGGTACT CGCACCTCTT GGCTTTGTTG ATGCTCCGCC AGGAAGGC
4651  CTTGTGTGTG CGTGTCAGTT ACTTTTTTAG TAACAATTCA GATCCAGT
4701  AAACTTCCGT TCATTGCTCT CCAGTCACAT GCCCCCACTT CCCCACAG
4751  GAAAGTTTTT CTGAAGTGTT GGGATTGGTT AAGGTCTTTA TTTGTATTA
4801  GTATCTCCCC AAGTCCTCTG TGGCCAGCTG CATCTGTCTG AATGGTGCG
4851  GAAGGCTCTC AGACCTTACA CACCATTTTG TAAGTTATGT TTTACATGC
4901  CCGTTTTTGA GACTGATCTC GATGCAGGTG GATCTCCTTG AGATCCTGA
4951  AGCCTGTTAC AGGAATGAAG TAAAGGTCAG TTTTTTTTGT ATTGATTTT
5001  ACAGCTTTGA GGAACATGCA TAAGAAATGT AGCTGAAGTA GAGGGGACG
5051  GAGAGAAGGG CCAGGCCGGC AGGCCAACCC TCCTCCAATG GAAATTCCC
5101  TGTTGCTTCA AACTGAGACA GATGGGACTT AACAGGCAAT GGGGTCCAC
5151  TCCCCCTCTT CAGCATCCCC CGTACC
```

FIG. 6A

MUM-1	(23-72)	KLRQWLIDQI	DSGKYPGLVW	ENEEKSIFRI	PWKHAGKQDY	NREEDAALFK
LSIRF	(23-72)	KLRQWLIDQI	DSGKYPGLVW	ENEEKSVFRI	PWKHAGKQDY	NREEDAALFK
IRF-1	(7-56)	RMRPWLEMQI	NSNQIPGLIW	INKEEMIFQI	PWKHAAKHGW	DINKDACLFR
IRF-2	(7-56)	RMRPWLEEQI	NSNTIPGLKW	LNKEKKIFQI	PWMHAARHGW	DVEKDAPLFR
ICSBP	(9-60)	RLRQWLIEQI	DSSMYPGLIW	ENEEKSMFRI	PWKHAGKQDY	NQEVDASIFK
ISGF3γ	(11-60)	KLRNWVVEQV	ESGQFPGVCW	DDTAKTMFRI	PWKHAGKQDF	REDQDAAFFK
IRF-3	(7-55)	RILPWLVSQL	DLGQLEGVAW	VNKSRTRFRI	PWKHGLRQD.	AQQEDFGIFQ

MUM-1	(73-122)	AWALFKGKFR	EGIDKPDPPT	WKTRLRCALN	KSNDFEELVE	RSQLDISDPY
LSIRF	(73-122)	AWALFKGKFR	EGIDKPDPPT	WKTRLRCALN	KSNDFEELVE	RSQLDISDPY
IRF-1	(57-106)	SWAIHTGRYK	AGEKEPDPKT	WKANFRCAMN	SLPDIEEVKD	QSRNKGSSAV
IRF-2	(57-106)	NWAIHTGKHQ	PGVDKPDPKT	WKANFRCAMN	SLPDIEEVKD	KSIKKGNNAF
ICSBP	(59-107)	AWAVFKGKFK	EG.DKAEPAT	WKTRLRCALN	KSPDFEEVTD	RSQLDISEPY
ISGF3γ	(61-109)	AWAIFKGKYK	EG.DTGGPAV	WKTRLRCALN	KSSEFKEVPE	RGRMDVAEPY
IRF-3	(56-104)	AWAEATGAYV	PGRDKPDLPT	WKRNFRSALN	RKEGLRLAED	RSK.DPHDPH

MUM-1	(123-130)	KVYRIVPE
LSIRF	(123-130)	KVYRIVPE
IRF-1	(107-114)	RVYRMLPP
IRF-2	(107-114)	RVYRMLPL
ICSBP	(108-115)	KVYRIVPE
ISGF3γ	(110-117)	KVYQLLPP
IRF-3	(105-112)	KIYEFVNS

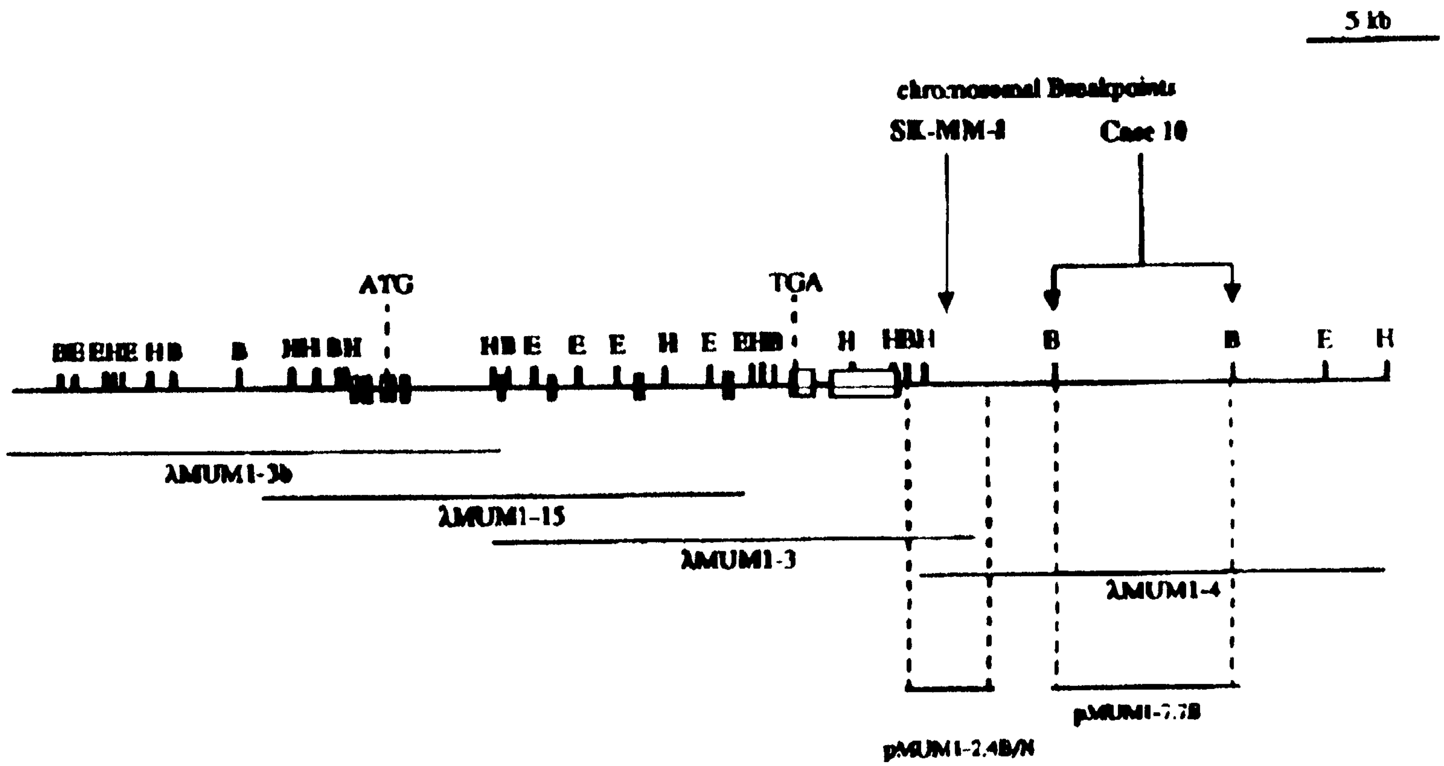
FIG. 6B

```
MUM-1  (327-372) KRLCQSTIYW DGPLAL.... CNDRPNKLER DQTCKLFDTQ QFLSELQAFA
LSIRF  (327-372) KRLCQSRIYW DGPLAL.... CSDRPNKLER DQTCKLFDTQ QFLSELQVFA
ICSBP  (289-334) KRLCQGRVFC SGNAVV.... CKGRPNKLER DEVVQVFDTS QFFRELQQFY
ISGF3γ (290-335) QRLCPIPISW NAPQAP.... PGPGPHLLPS NECVELFRTA YFCRDLVRYF
IRF-3  (284-333) QRLGHCHTYW AVSEELLPNS GHGPDGEVPK DKEGGVFDLG PFIVDLITFT

MUM-1  (373-421) HHGRSLPRFQ VTLCFGEEFP DPQRQR.KLI TAHVEPLLAR QLYYFAQQNS
LSIRF  (373-421) HHGRPAPRFQ VTLCFGEEFP DPQRQR.KLI TAHVEPLLAR QLYYFAQQNT
ICSBP  (335-384) NSQGRLPDGR VVLCFGEEFP DMAPLRSKLI LVQIEQLYVR QLAEEAGKSC
ISGF3γ (336-385) QGLGPPPKFQ VTLNFWEESH GSSHTPQNLI TVKMEQAFAR YLLEQTPEQQ
IRF-3  (334-383) EGSGRSPRYA LWFCVGESWP QDQPWTKRLV MVKVVPTCLR ALVEMARVGG
```

FIG. 7

Genomic locus spanning MUM1 gene



pMUM1- 7.7 B means the 7.7kb insert in BamHI sites .

B/N means BamHI/NotI site is used for cloning.

Every genomic inserts is cloned into pBluescript KS.

FIG. 8

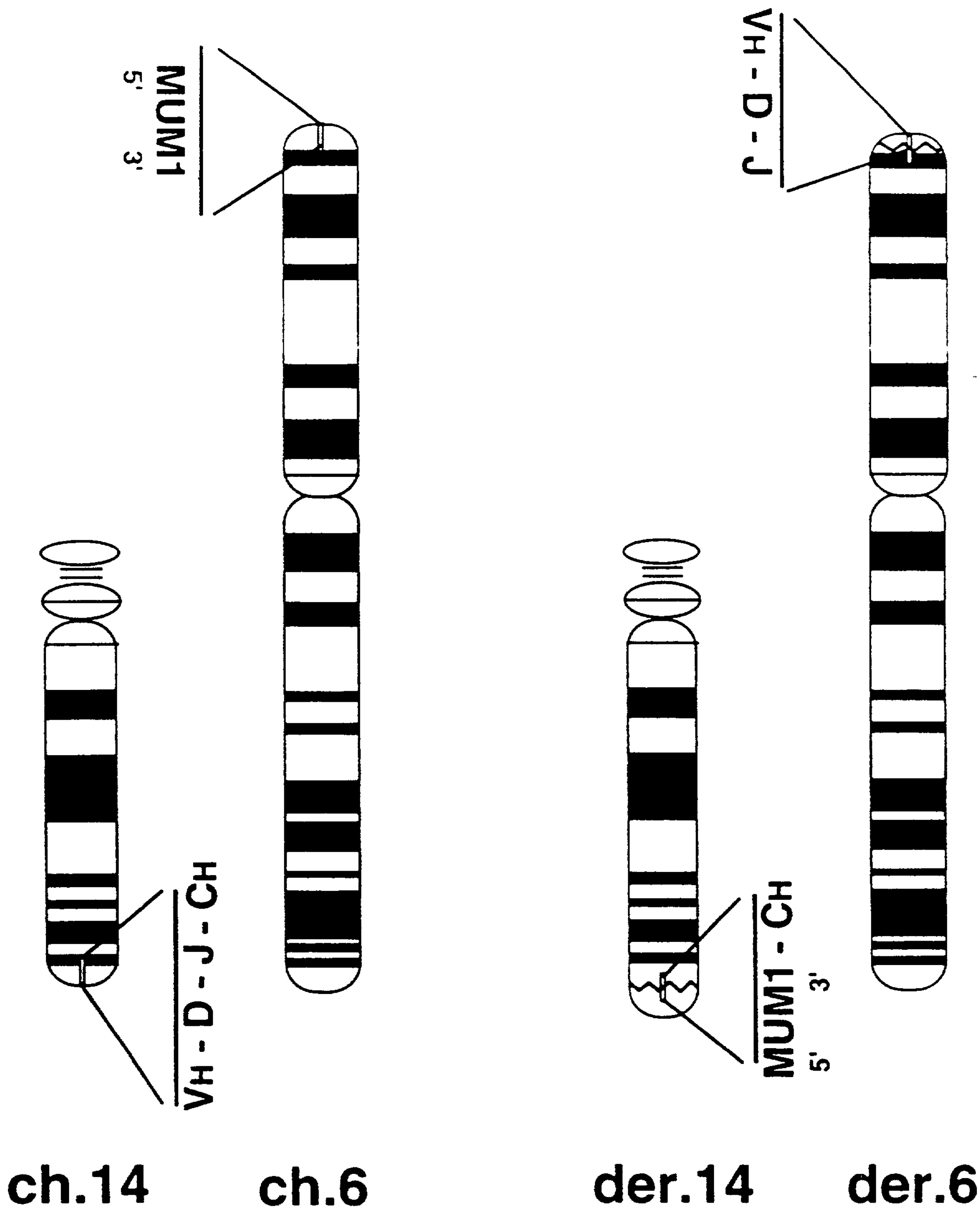


FIG. 9A

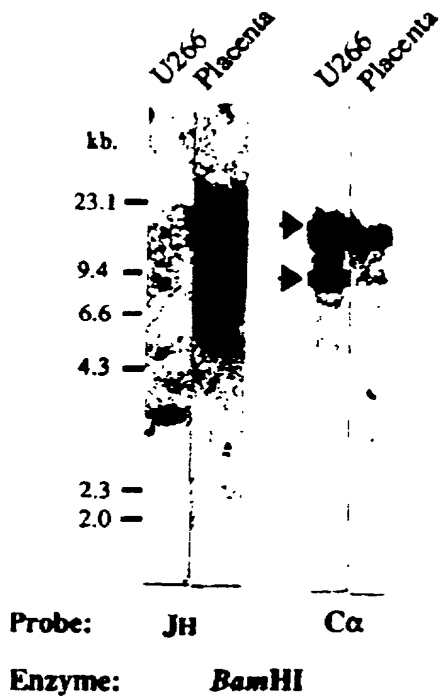
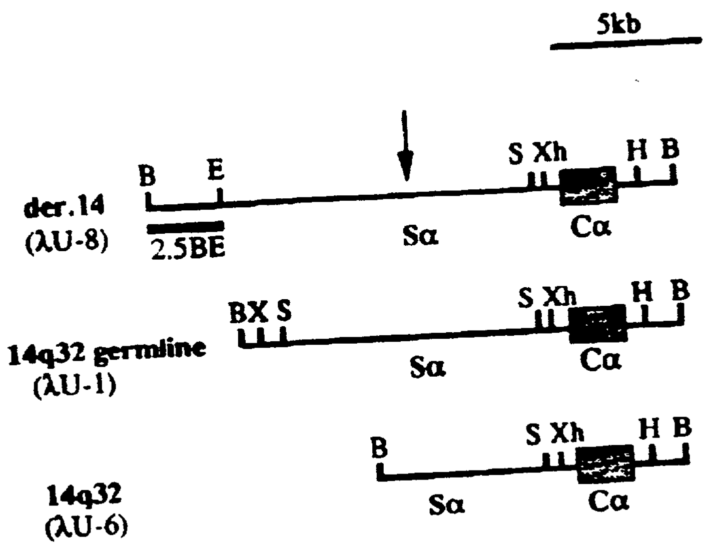

U266
Placenta
U266
Placenta
kb.
23.1
9.4
6.6
4.3
2.3
2.0
Probe:
JH
Cα
Enzyme:
BamHI

FIG. 9B



B, *Bam*HI; H, *Hind*III; S, *Sac*II; X, *Xba*I;
Xh, *Xho*I

chromosomal breakpoint

FIG. 10

MUM2 Transcripts detected in MM/PCL Cell Lines

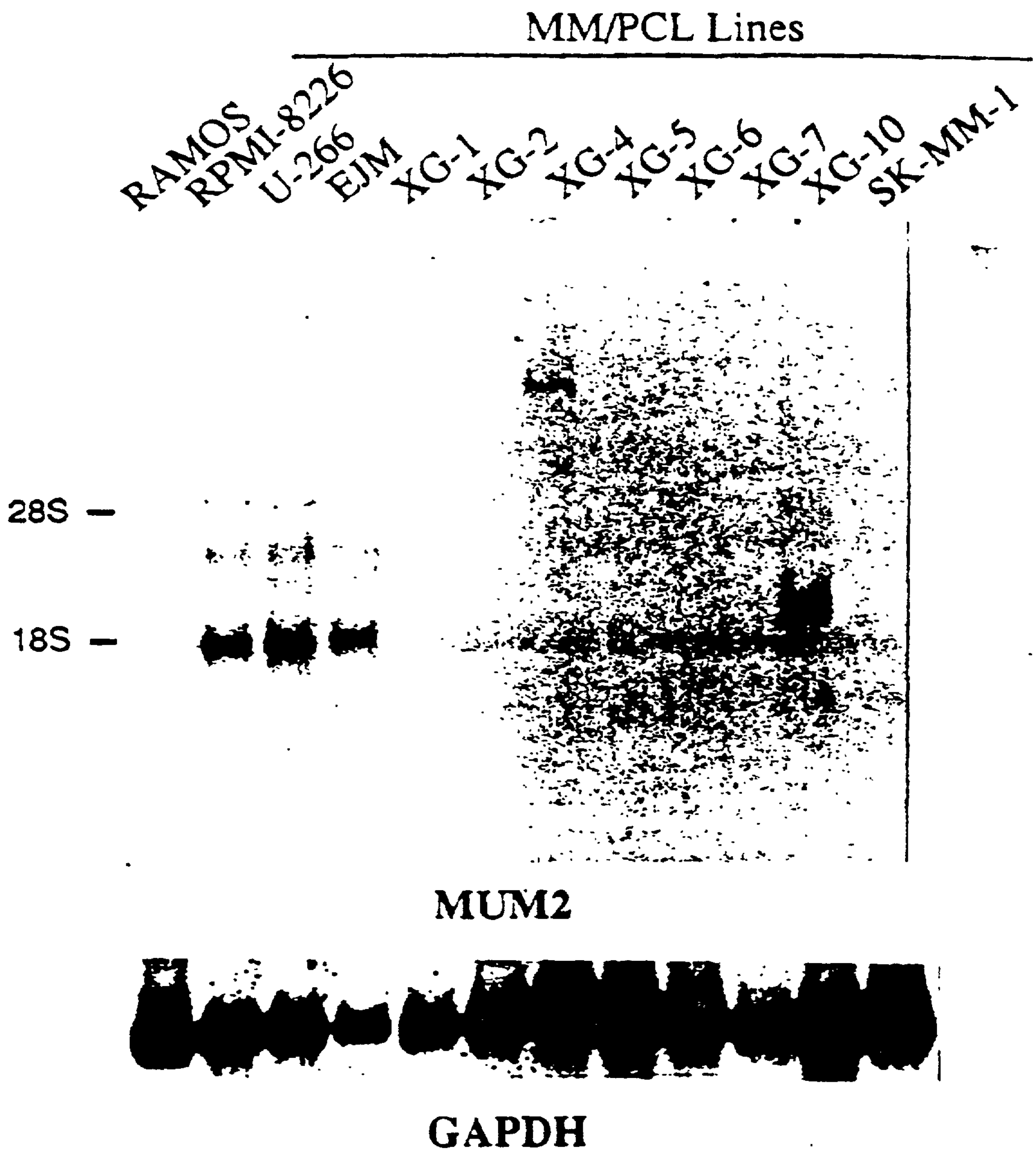


FIG. 11A

Physiological IgH gene rearrangement

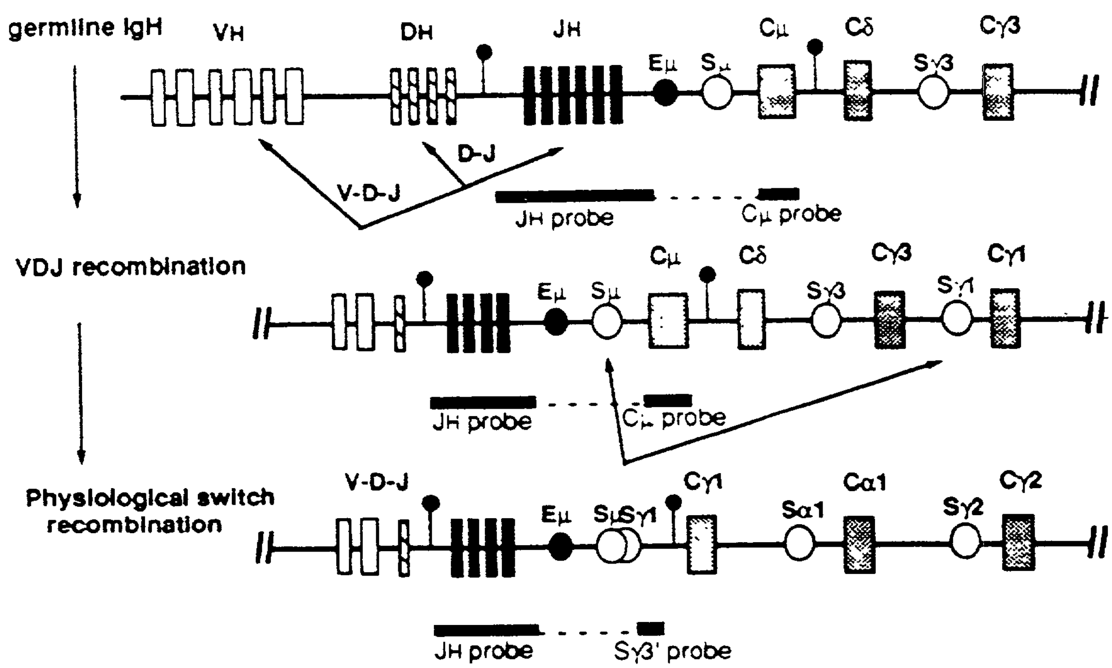
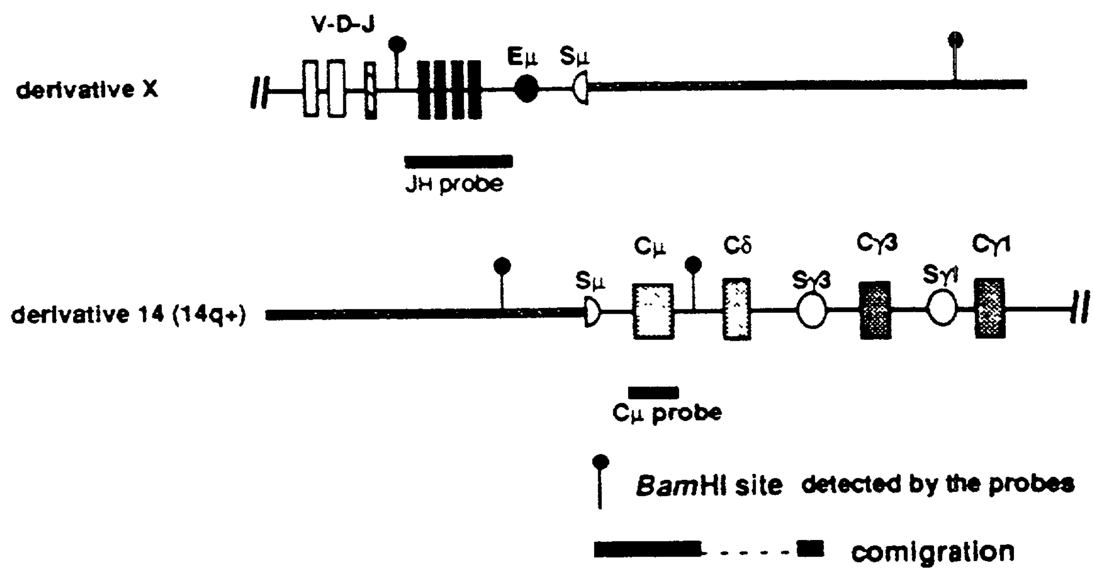
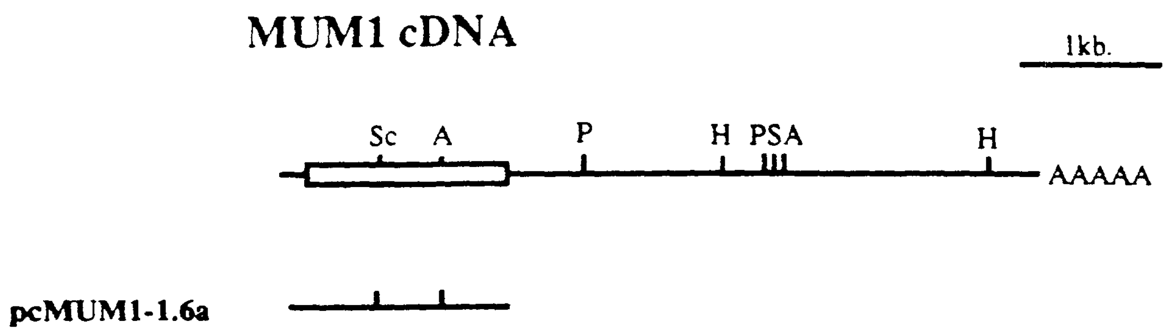


FIG. 11B

Chromosomal translocation occuring in switch region





Sc; SacII, P;*Pst*I, H;*Hin*dIII, S;*Sac*I, A;*Apa*I

cDNA inserts is cloned into EcoRI / BamHI site of the pBluescript KS+ Bacteria strain used is DH5α cells. pcMUM1-1.6a contains full length open reading frame of nt.217~1572.

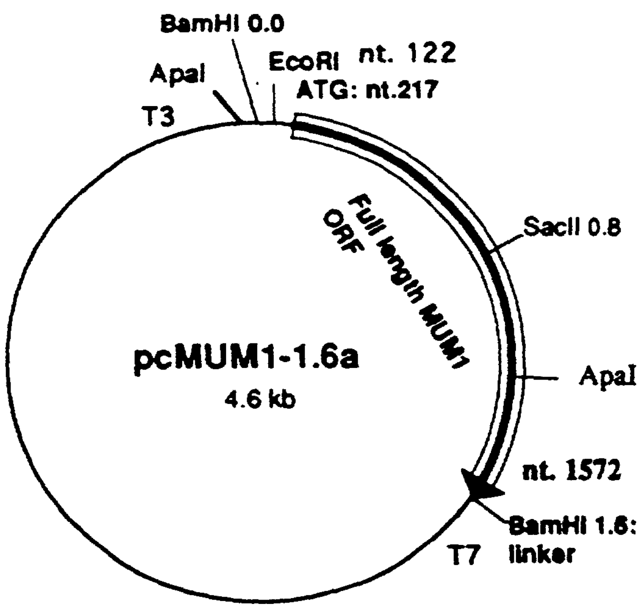
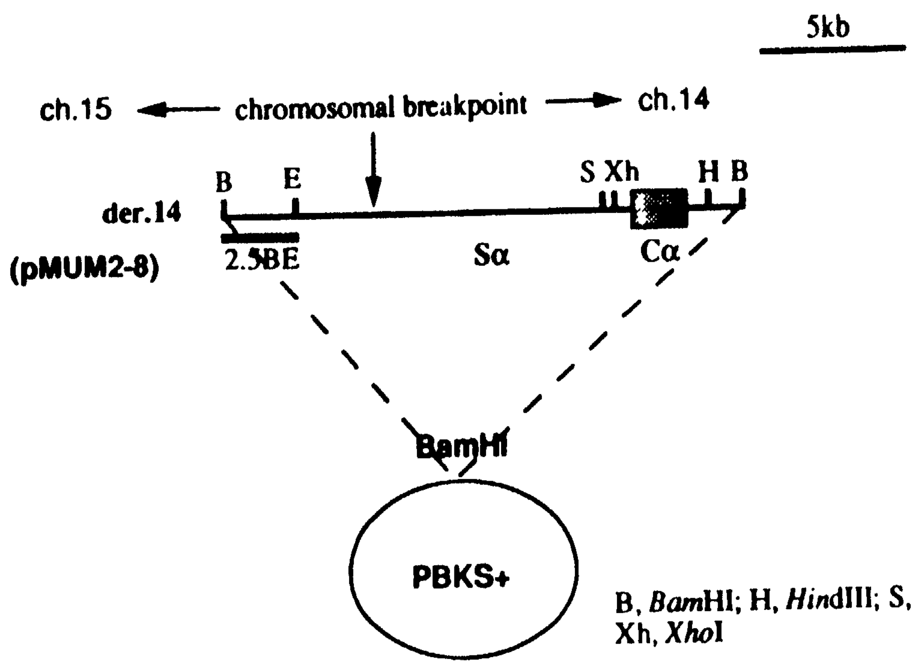


FIG. 12 A-B

Breakpoint Cloning of the U-266 Cell Line



B, *Bam*HI; H, *Hin*dIII; S, *Sac*II; X, *Xba*I;
Xh, *Xho*I

pMUM2-8 has a 22.0kb insert in BamHI site of pBluescript KS+.

FIG. 13

DECLARATION AND POWER OF ATTORNEY

As a below-named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled.

IDENTIFICATION OF GENES ALTERED IN MULTIPLE MYELOMA

the specification of which:
(check one)

___ *is attached hereto.*

X *was filed on* May 28, 1996 *as*

Application Serial No. Not Yet Known

and was amended May 28, 1996.
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 (a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International Application which designated at least one country other than the United States, listed below. I have also identified below any foreign application for patent or inventor's certificate, or PCT International Application having a filing date before that of the earliest application from which priority is claimed:

Prior Foreign Application(s) — *Priority Claimed*

Number	Country	Filing Date	Yes	No
N/A				

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below:

Provisional Application No.	*Filing Date*	*Status*

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States Application(s), or Section 365(c) of any PCT International Application(s) designating the United States listed below. Insofar as this application discloses and claims subject matter in addition to that disclosed in any such prior Application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56, which became available between the filing date(s) of such prior Application(s) and the national or PCT international filing date of this application:

Application Serial No.	*Filing Date*	*Status*

And I hereby appoint

John P. White (Reg. No. 28,678); Thomas F. Moran (Reg. No. 16,579); Norman H. Zivin (Reg. No. 25,385); Ivan S. Kavrukov (Reg. No. 25,161); Christopher C. Dunham (Reg. No. 22,031); Thomas G. Carulli (Reg. No. 30,616); Robert D. Katz (Reg. No. 30,141); Peter J. Phillips (Reg. No. 29,691); Richard S. Milner (Reg. No. 33,970); Albert Wai-Kit Chan (Reg. No. 36,479); Kristina L. Konstas (Reg. No. 37,864); Mary Anne P. Tanner (Reg. No. 40,197); Timothy X. Witkowski (Reg. No. 40,232); and Mary Catherine DiNunzio (Reg. No. 37,306)

and each of them, all c/o Cooper & Dunham LLP, 1185 Avenue of the Americas, New York, New York 10036, my attorneys, each with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, to transact all business in the Patent and Trademark Office connected therewith and to file any International Applications which are based thereon under the provisions of the Patent Cooperation Treaty.

Please address all communications, and direct all telephone calls, regarding this application to:

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Cooper & Dunham LLP
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New York, New York 10036
Tel. (212) 278-0400

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true: and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Inventor's signature ______

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Post Office Address same as residence

Full name of joint inventor (if any) ______

Inventor's signature ______

Citizenship ______ *Date of signature* ______

Residence ______

Post Office Address ______

Full name of joint inventor (if any) ______

Inventor's signature ______

Citizenship ______ *Date of signature* ______

Residence ______

Post Office Address ______

50995-B/JPW/EMW

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Riccardo Dalla-Favera
U.S. Serial No.:		Not Yet Known (Continuation of U.S. Serial No. 08/654,482, filed May 28, 1996)
Filed	:	Herewith
For	:	IDENTIFICATION OF GENES ALTERED IN MULTIPLE MYELOMA

1185 Avenue of the Americas
New York, New York 10036
June 1, 2000

Assistant Commissioner for Patents
Washington, D.C. 20231

Box: Patent Application

SIR:

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